

EFFECT OF STORAGE IN A HOUSEHOLD REFRIGERATOR
ON THE ASCORBIC ACID CONTENT OF VEGETABLES

BY

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INTRODUCTION

Vegetables are essential in the daily diet because of their contribution of minerals and vitamins. They are a valuable source of vitamin C and therefore it is important to know how the handling of vegetables affects their vitamin C content. Because of the enzymes they contain, vegetables are sensitive to changes during storage. The action of these enzymes may be speeded up or retarded by increasing or lowering storage temperatures. This investigation was undertaken to study the effect of storage in a household refrigerator on the ascorbic acid content of vegetables. Those vegetables which are most commonly used and stored were used for analysis.

A new refrigerator which includes a moist chamber especially designed for vegetable storage has recently been placed on the market for household use. Figure 1 is a photograph of the refrigerator showing the interior. The glass doors near the bottom enclose the moist chamber where the vegetables were stored. This storage chamber was designed by the manufacturers primarily for storage of vegetables and other naturally moist foods where high humidity is desirable.

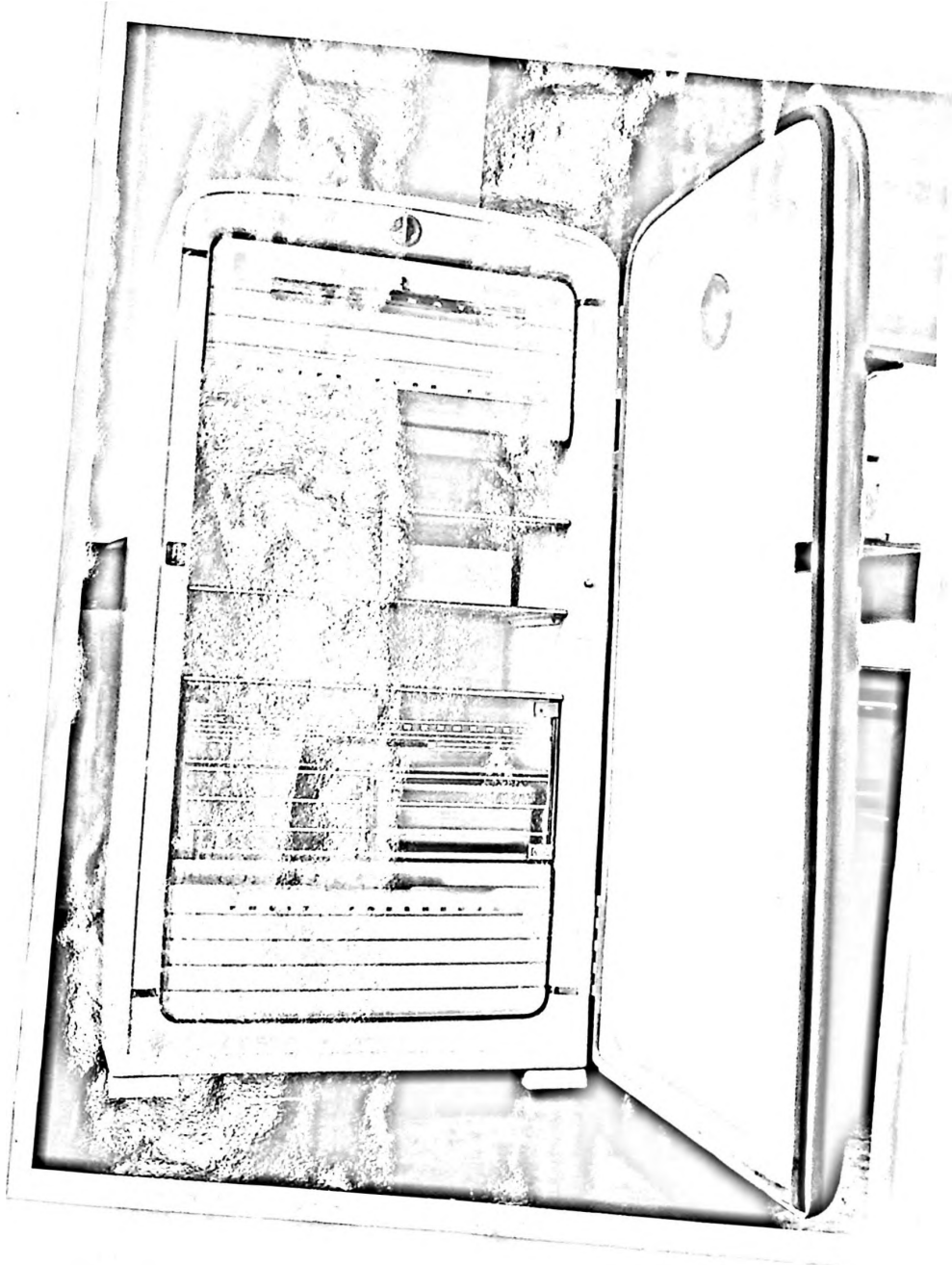


Figure 1. Refrigerator used for storage of vegetables

REVIEW OF LITERATURE

Identification of Ascorbic Acid.

When vitamin C was isolated it was found to be an organic acid. Before its isolation it was known only as vitamin C or the anti-scorbutic vitamin. In medical literature it is sometimes called cevitamic acid. Actually ascorbic acid is 2,3 dieneol-1-gulonic acid. Zilva (8) was among the early investigators to attempt the isolation of ascorbic acid. In his experiments to separate the vitamin from lemon juice, he found it increasingly evident that oxidation rapidly destroyed the potency of the vitamin. Later Szent-Györgyi isolated a white crystalline substance from adrenal glands. He called this strongly reducing compound hexuronic acid. In 1932 various groups of workers including Tilmanns (26) and Waugh and King (34) discovered that Szent-Györgyi's hexuronic acid and Zilva's reducing factor were both vitamin C, and they found that the vitamin could be reversibly oxidized and reduced.

Forms of Ascorbic Acid.

Ascorbic acid exists in two forms, reduced and dehydroascorbic acid. Both forms are believed to be biologically active (23). Dehydroascorbic acid is formed when reduced ascorbic acid is treated with a suitable oxidizing agent. It is formed naturally in foods by enzyme oxidation. Two atoms of hydrogen are lost from a molecule of reduced ascorbic acid when it is oxidized to dehydroascorbic acid. This reaction is reversible since one molecule of dehydroascorbic acid will take on two hydrogen atoms when treated with a reducing agent. However, all of the oxidized form cannot be obtained again

in the reduced form because some of the dehydroascorbic acid is usually converted to diketogulonic acid. Penny and Zilva (23) found that in vivo and in vitro the dehydroascorbic acid tends to be converted to diketogulonic acid with comparative ease both in the presence and in the absence of oxygen, the rate being influenced by the pH of the medium. These reactions occur only when ascorbic acid is in solution. The dry crystals are stable on exposure to air and daylight at ordinary room temperatures for long periods of time (2).

Figure 2 shows the structural formulae of reduced ascorbic, dehydroascorbic acid, and diketogulonic acid. The first reaction is reversible and both the reduced and dehydro form of ascorbic acid are believed to be equally utilized by the body (23). The second reaction is not reversible and this form is not believed to be utilized by the body (23).

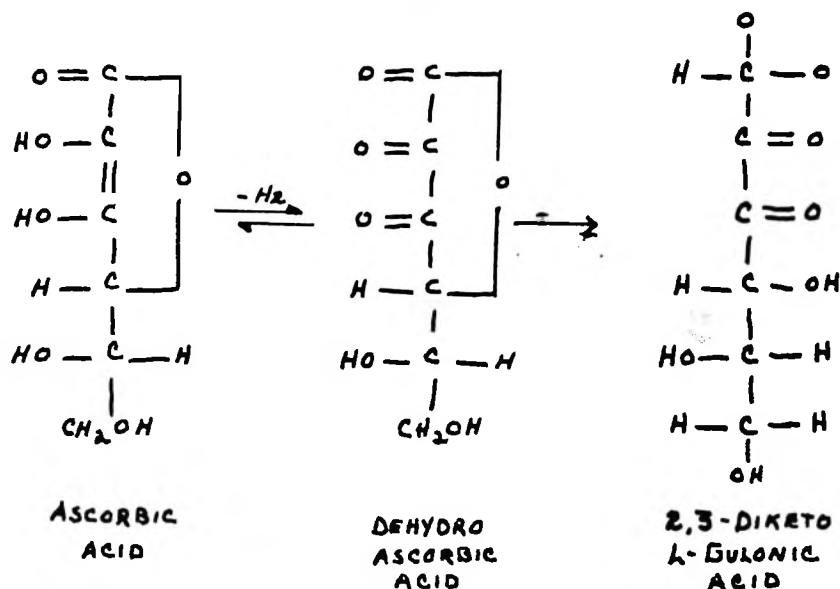


Figure 2. Structural formulae of reduced ascorbic acid, dehydroascorbic acid, and diketogulonic acid showing reversible and irreversible reactions.

Some investigators have not considered that determinations of the dehydro form of ascorbic acid essential, but a number of workers have published data for this biologically active form. Harris (12) reported that the quantity of dehydroascorbic acid normally found in fruits and vegetables was so small as to be of little practical significance. He believed that the relatively large amounts of dehydroascorbic acid reported by some investigators could be attributed to a failure to guard against oxidation during the preparation of the extract. Hochberg (14), however, stressed the importance of determining dehydroascorbic acid. Results from a study on storage of dehydrated tomato soup indicated that failure to determine dehydroascorbic acid made the loss of the vitamin appear to be much greater than that which had actually occurred. Hochberg (14) stated that dehydroascorbic acid may be present in foods at the time of analysis, or it may arise in the course of analysis from oxidation of reduced ascorbic acid. Atmospheric oxidation which is accelerated by oxidases and copper, especially with increasing temperatures must be prevented during analysis if a reliable value for reduced ascorbic acid is to be obtained. Penny and Zilva (23) used thiourea in metaphosphoric acid to stabilize the ascorbic acid during the extraction and subsequent treatment of plant tissue.

Analyzing Plant Tissue For Ascorbic Acid.

The successful measurement of ascorbic acid in plants is largely dependent upon the quick removal of the vitamin from the tissue to a protecting solution. Oxidation does not rapidly occur when the vitamin is in its natural environment which contains protective anti-oxidants. Glutathione which is present in active plant tissues

has a strong reducing reaction and is a protective agent for vitamin C in plant cells and animal tissues. There is an enzyme which produces the opposite reaction, that is the oxidation of vitamin C, and this enzyme is called ascorbic acid oxidase; it is a respiratory enzyme. All analyses for ascorbic acid should be carried out in the absence of oxygen, in the absence of copper which catalyzes the oxidation, and in subdued light. For the present study all analyses were made with reagents prepared with glass-redistilled water which gave a negative test for copper with sodium diethyldithiocarbamate. The extracting mechanism was a Waring blender and its speed was cut to approximately one-half normal by the use of a variable resistor. Decreasing the speed helped to minimize incorporation of air which would result in increased oxidation. All blender jars were covered with plastic tops.

Ascorbic acid is a strong reducing agent and its capacity to reduce substances such as silver nitrate, iodine, ferricyanide, and methylene blue has served as the basis for qualitative and quantitative methods for its analysis. The most widely used reagent for the determination of ascorbic acid has been sodium 2,6-dichloro-benzinoneindophenol. This dye was introduced by Tillmanns and his coworkers and is sometimes called the Tillmanns reagent. This dye is blue in alkaline solutions and red in acid solutions. It is immediately reduced by ascorbic acid to a colorless compound and can be used for titration of ascorbic acid without the use of any other indicator.

The method used in this study for the determination of reduced ascorbic acid was first described by Mindlin and Butler (18).

Modifications were later made by Bessey (3), who made a correction so that the method could be adapted to make measurements in colored or turbid extracts, and Morrell (20), who used a Waring Blendor for the extraction and filtered the extract instead of using a centrifuge. One of the first methods used was the visual titration method based on the reduction of sodium 2,6-dichlorobenzinoneindophenol by an acid solution of ascorbic acid. The method described by Mindlin and Butler uses a photometric apparatus which eliminates eye judgement of the end-point which was necessary in the titration methods. The formulae for the oxidation of reduced ascorbic acid to dehydroascorbic acid by the dye is shown in figure 3. However, there may be other substances present in the solution being analyzed which are capable of reducing the dye, but these substances are slower in their reducing action especially in an acid solution of pH of 3.0 to 4.0. Analyses using metaphosphoric acid filtrates have been used by most investigators. With the aid of the photoelectric colorimeter and readings made at definite time intervals, the much faster reducing action by ascorbic acid can be determined before the interfering substances have had their effect.

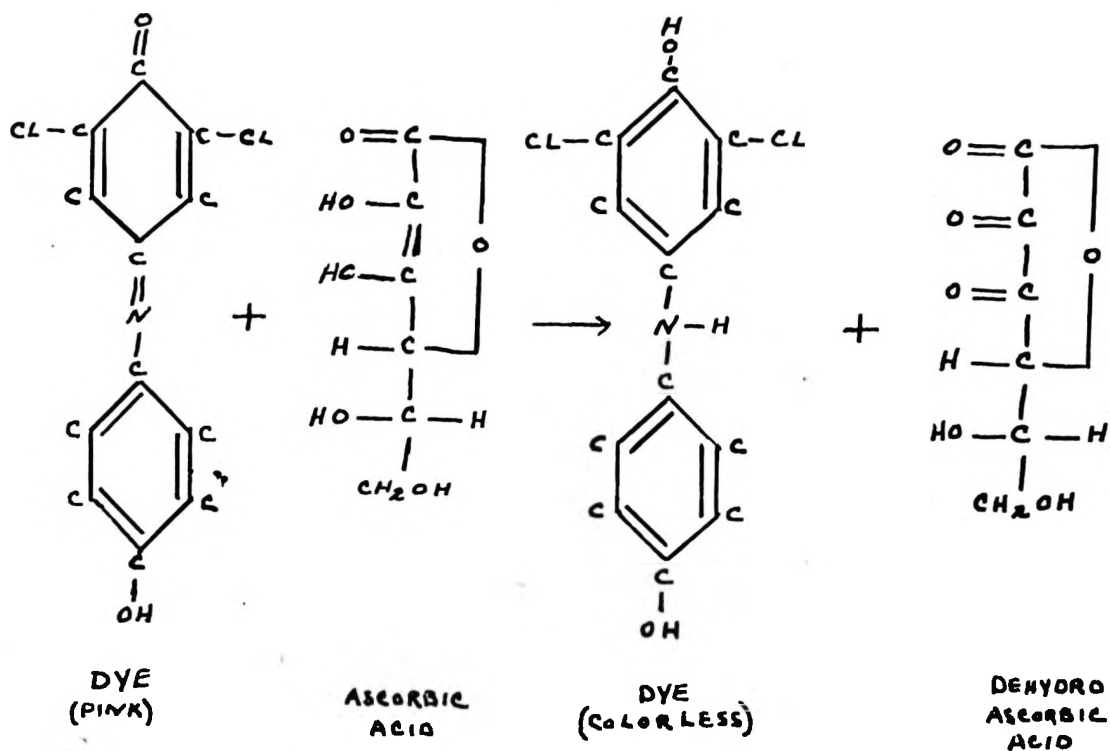


Figure 3. Reaction showing oxidation of reduced ascorbic acid to dehydroascorbic acid by sodium 2,6-dichlorobenzinonein-dophenol.

The pH of the filtrate is an important factor in quantitative determinations. Mindlin and Butler (19) have shown that the dye solution decomposes if the pH is too low when extracts are added. Therefore, a suitable pH must be chosen so that it will be high enough to prevent the spontaneous decomposition of the dye and low enough to prevent reduction by reducing substances other than ascorbic acid. A pH range of 3.5 to 3.6 was recommended by Bessey (3) as the most suitable.

In the present study determinations were also made of the amount of dehydroascorbic acid present. The procedure used was that

described by Roe and Oesterling (25). Ascorbic acid and dehydroascorbic acid enter into certain chemical reactions which are characteristic of sugars, such as the formation of osazones which are colored substances. Dehydroascorbic acid reacts with 2,4-dinitrophenylhydrazine to form a characteristic osazone. When treated with sulfuric acid this osazone is converted into a soluble red pigment. Therefore, the method described by Roe and Oesterling is based upon the measurement at 540 mu of the red color produced by this series of reactions carried out under carefully controlled conditions. 540 mu was the wavelength at which Roe reported the color could best be determined and where there would be the least interference from other substances.

Summary of Related Storage Studies.

Many studies have been reported on the effect of storage on the ascorbic acid content of vegetables. Harris and Wismann (13) reported a smaller loss of ascorbic acid when vegetables were stored in refrigerators which provided decreased air movement and increased humidity, both of which reduce evaporation. Their studies showed that this type of storage retarded wilting of the vegetable. It is customary for wilting plasymolysis or the breaking down of cell walls to accelerate the oxidation of the cell constituents and impair the natural resistance of the plant to microbial invasion.

Patton and Miller (22) at the Ohio Agricultural Experiment Station studied the use of crushed ice refrigeration in comparison with mechanical refrigeration and found that in every case the vegetables stored in crushed ice retained a higher percent of their ascorbic acid than those stored in a refrigerator at 40-50° F. They reported that cabbage, tomatoes, and green peppers retained 94, 88,

and 85 percent respectively of their original ascorbic acid content when held on top of crushed ice at room temperature for forty-eight hours. All other vegetables studied showed a much lower percent retention under this storage condition.

Spinach: Zeppelin and Elvehjem (37) reported that spinach lost as much as fifty-five percent of its ascorbic acid after three days of storage in the hydrator of an electric refrigerator. Gleim, Tressler, and Fenton (10) reported a loss of thirty-five percent in spinach after storage for one week at 4° C. Ranganathan (24) reported losses of thirty-four to forty-seven percent of ascorbic acid caused by storage of spinach at room temperature for twenty-four hours, and a loss of ninety-five percent after eight days. Tressler, Mack, and King (31) found that when spinach was stored at room temperature for three days it lost fifty percent of its ascorbic acid; when spinach was stored in a refrigerator at 33 to 37° F. for three days it retained nearly all of its original ascorbic acid. Olliver (21) reported a loss of seventy-eight percent from spinach held at room temperature for two days, while storage at 32° caused slightly less destruction. Rudra (27) noted a nineteen percent loss of ascorbic acid in spinach stored at 41° F. for twenty-four hours.

Tomatoes: Brown and Moser (6) stored tomatoes at 44° F. for eighteen days and found no indication of ascorbic acid loss. They found that small tomatoes were a more potent source of ascorbic acid than the larger tomatoes, and there was some evidence that the vitamin C content increased with advance of the season.

Green beans: Van Duyne and Chase (33) worked with two varieties of green beans and made moisture and ascorbic acid determinations within

approximately two hours after harvesting. These analyses were repeated after holding for different periods of time at room temperature and at 35-40° F. in a refrigerator. They found no significant difference between the amounts of ascorbic acid present within approximately two hours after harvesting and after holding for one day in the refrigerator. Holding the beans at room temperature resulted in significant decreases in the ascorbic acid content. Compared on the dry weight basis Bountiful snap beans lost thirty-one percent of their ascorbic acid content, and Stringless Black Valentine snap beans lost thirty percent in twenty-four hours. Zeppelin and Elvehjem (37) reported that green beans lost twenty percent of their ascorbic acid when held for twenty-four hours at room temperature and ten percent if kept on the shelf of a mechanical refrigerator for forty-eight hours. Mack, Tapley, and King (17) studied the effect of storage on the ascorbic acid content of four varieties of snap beans held at three different temperatures. Their results showed that in some instances variety might be a more important factor than temperature in determining the amounts retained. This might explain the variations in the percent retentions reported by different workers.

Feener, Palmer, and Fitzgerald (9) at the Birdseye Laboratory in Boston reported that there was no appreciable loss in ascorbic acid content of spinach or green beans after two days storage at 1 to 3° C. Analyses were repeated each month over a period of a year on vegetables secured from a local market. Results showed a great variation in the amount of ascorbic acid in the spinach and green beans during the various seasons.

Cabbage: Gould and Tressler (11) stored cabbage as long as eighty-four days at 46-48° F. and reported a loss of twelve milligrams per one hundred grams at the end of the storage period.

In 1946 McMillan and Todhunter (18) reported that Flat Dutch cabbage contained 47.3 mg. of reduced ascorbic acid per 100 grams and 4.3 mg. of dehydroascorbic acid per 100 gm. of cabbage. After standing for two hours at room temperature the reduced ascorbic acid content was 40.9 mg. per 100 gm., but the dehydroascorbic acid content had increased to 8.5 mg. per 100 gm. of cabbage.

METHOD

Type of Storage.

An electric refrigerator*, eight and one-half cubic foot capacity, was used for the storage of the vegetables. One section of the refrigerator was designed for vegetable storage (figure 1). This compartment was sealed off from the remaining chest by glass shelves and glass doors to retard air circulation and prevent evaporation. It was cooled by a set of coils inside the walls. Dials at the rear corners of an adjustable baffle under the frozen food chest were provided to regulate the flow of air into this storage compartment. This was designed to increase or decrease the moisture content of the air inside the chamber and provide favorable storage conditions for vegetables and other naturally moist foods.

Inside this moist chamber was an aluminum vegetable pan in which the vegetables were stored. The vegetables were arranged so that they would not be touching each other. When more than one vegetable was being stored and there was not enough room inside the vegetable pan, the overflow was placed on the glass shelves inside the moist chamber.

The temperature control was placed at the normal setting recommended by the manufacturer for average household use. This setting gave a temperature which averaged 38-42° F. inside the storage chamber. This temperature varied according to the room temperature and to the number of times the door was opened.

* Acknowledgment is made to the Nash Kelvinator Corporation, Detroit, Michigan, for the use of their refrigerator (Model MM-48).

A record was kept of the temperature inside the storage chamber during the storage of all vegetables. This temperature recording was made by the use of a Bristol Temperature Recording Instrument Model 144 which was placed on the glass shelf inside the storage chamber. This portable recording instrument held a four-inch chart which would record temperatures ranging from 20° to 60° F. over a twenty-four hour period. Figure 4 illustrates the chart used and the recording made on 7/14/48. The recorder was removed from the refrigerator while the chart was being changed each day, and one to three hours were required before the recorder was again actually recording the temperature inside the chamber. An increased temperature was recorded on the chart when the instrument had been exposed to room temperature while the chart was being changed. The higher temperatures recorded during this time were not included when the average temperature of 38 to 42° F. was determined.

A record was also kept of the humidity inside of the storage chamber during the storage of all vegetables. A Bristol Humidigraph Model 4044 was used to record the humidity inside the storage chamber. The portable humidigraph was placed with the temperature recording instrument on the glass shelf inside the storage chamber. It also held a four-inch chart which would record humidity ranging from 10 to 90 percent over a twenty-four hour period. When there was an excess humidity inside the storage chamber the baffle under the frozen food chest could be lowered by adjusting the dials. Lowering the baffle admitted more air to the surface of the frozen food chest which reduced the amount of moisture in the air. Figure 5 illustrates

the chart used in the humidigraph and the recording made on 7/14/48. The humidigraph was removed from the refrigerator while the chart was being changed each day, and from one to three hours were required before the humidigraph was again actually recording the humidity inside the storage chamber. The accuracy of the humidigraph was checked with a sling type hygrometer.

This study was designed to determine the effect of household refrigeration on vegetables, therefore the length of the storage periods were planned on the basis of the length of time the average homemaker might store vegetables. Most fresh vegetables which can be kept in the refrigerator are used within a week after purchase, therefore, seven days was chosen as the maximum storage period.

All vegetables were analyzed on the day of purchase and two days later another analysis was made. Four and seven days after the day of purchase analyses were repeated. These storage periods were believed to be representative of the different periods of time a homemaker might use for vegetable storage.

Selection and Purchasing of Vegetables.

The vegetables chosen for analysis were those which were representative of the types usually stored in a household refrigerator and those which are widely used throughout the country. The amount of ascorbic acid present in the vegetables was also a determining factor in the choice of vegetables to be analyzed, and those vegetables containing appreciable amounts of ascorbic acid were chosen for this study.

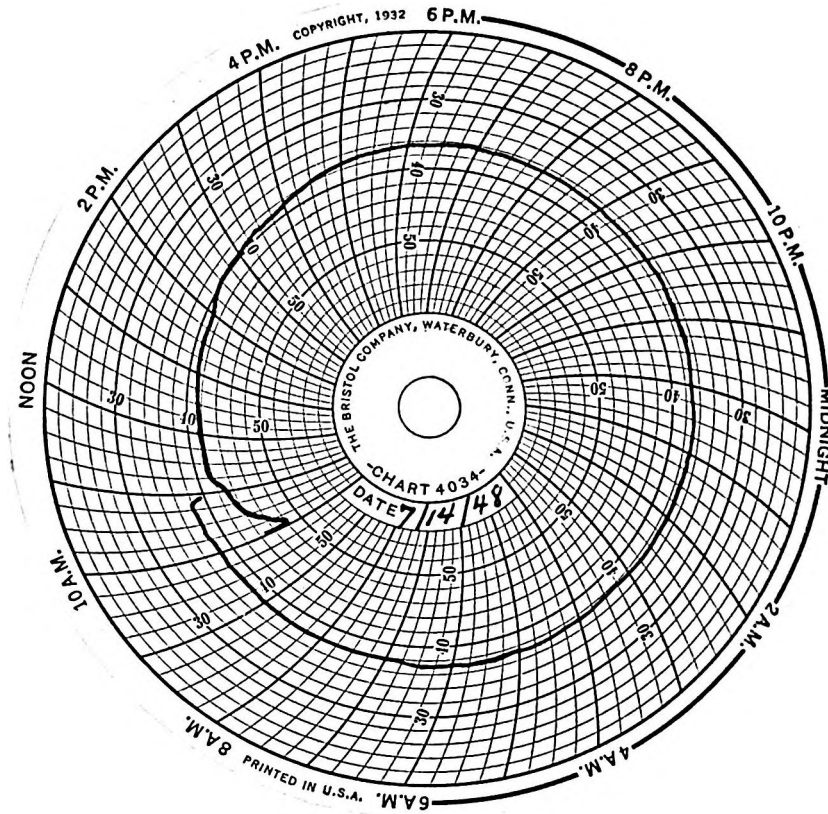


Figure 4. Temperature chart and recording made on 7/14/48.

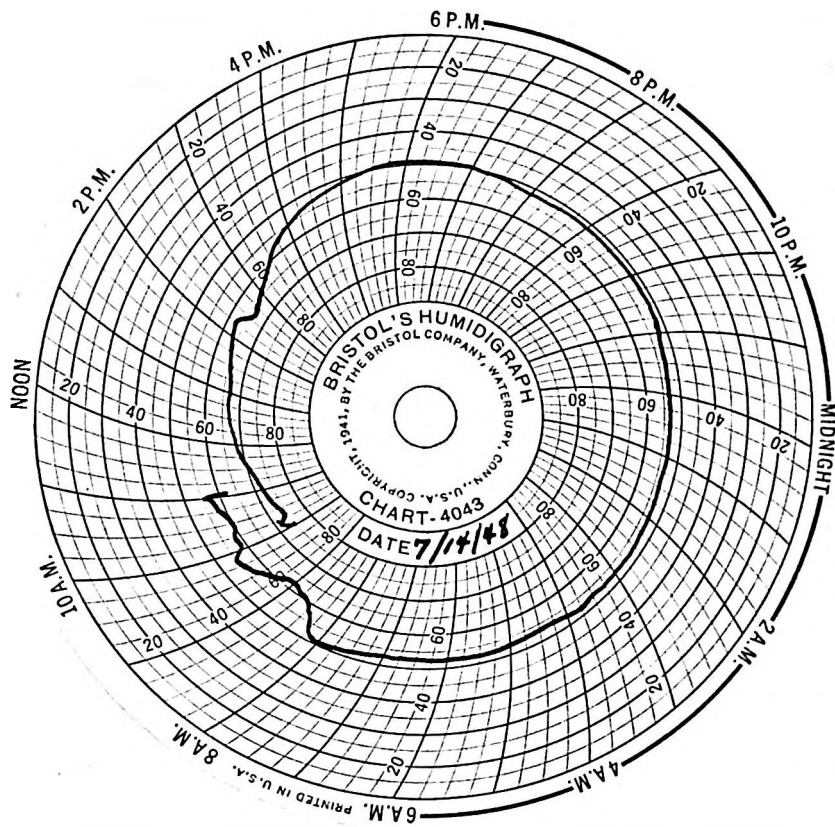


Figure 5. Humidigraph chart and recording made on 7/14/48.

Table 1
DESCRIPTION OF VEGETABLES ANALYZED

Vegetable	Variety	Date analyzed
Cabbage	Large Jersey Wakefield	June 1948
	Small Jersey Wakefield	June 1948
	Large Flat Dutch	October 1948
	Small Jersey Wakefield	September 1948
Spinach	Aragon curly leaf	October 1948
Green beans	Pole	November 1948
Green peppers	Bell	August 1948
Tomatoes	Marglobe	July 1948

Table 2
VEGETABLES ANALYZED AND DATE OF PURCHASE

<p>Summer Cabbage Large heads</p> <ul style="list-style-type: none"> a. June 7, 1948 b. June 21, 1948 c. July 5, 1948 d. July 5, 1948 e. July 26, 1948 <p>Small heads</p> <ul style="list-style-type: none"> a. June 22, 1948 b. June 22, 1948 c. June 30, 1948 d. June 30, 1948 e. July 14, 1948 <p>Fall Cabbage Large heads</p> <ul style="list-style-type: none"> a. October 11, 1948 b. October 11, 1948 c. October 25, 1948 d. October 25, 1948 e. October 26, 1948 <p>Small heads</p> <ul style="list-style-type: none"> a. September 28, 1948 b. September 28, 1948 c. September 29, 1948 d. September 29, 1948 e. November 3, 1948 	<p>Tomatoes Sample</p> <ul style="list-style-type: none"> a. July 13, 1948 b. July 19, 1948 <p>Green Peppers Sample</p> <ul style="list-style-type: none"> a. August 2, 1948 b. August 9, 1948 <p>Spinach Sample</p> <ul style="list-style-type: none"> a. October 14, 1948 b. October 26, 1948 c. November 4, 1948 d. November 29, 1948 <p>Green Beans Sample</p> <ul style="list-style-type: none"> a. November 8, 1948 b. November 9, 1948 c. November 30, 1948 d. December 2, 1948
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Five vegetables were chosen for analysis. These were typical of different parts of plants which are used as vegetable foods and included leaf, head, pod, and fruit of the plant.

Cabbage was chosen for the head vegetable. Since size and season might cause a difference in the ascorbic acid content, two series of large and small cabbage were analyzed. The first series of analyses began in June 1948, and the second series in October 1948. The range in weight for the large heads was from 527 grams to 591 grams. The mean weight of the large heads was 560 grams. The range in weight for the small heads was from 385 to 431 grams. The mean weight for the small heads was approximately 410 grams. The variety of both large and small heads used in the June study was the Jersey Wakefield. This variety had been shipped in from South Carolina. In October the variety of the large cabbage was the Flat Dutch; the variety of the small cabbage was Jersey Wakefield. Both varieties used in the October study were grown in Wisconsin. All cabbage arrived at the grocery store in burlap bags and had not been refrigerated enroute or in the grocery store.

Green beans and green peppers were chosen for the pod vegetables. The green beans were the pole variety and had been grown in Florida. The beans had been shipped in wooden hampers via motor express without refrigeration but had been under refrigeration at the store for two days before purchase. Analyses were made in November 1948.

Green peppers were analysed in August 1948 when they were in season in Alabama. The peppers were locally grown and were the Bell variety. They were brought to the store in market baskets without

refrigeration. The peppers used for the study were purchased the same morning they were brought to the store.

Spinach was chosen for the leafy vegetable. It was analyzed in October 1948 and the Aragon Curly leaf was the variety used. The spinach was grown in an adjacent county and brought to the grocery store without being refrigerated. It was purchased soon after being delivered to the store and was still quite crisp and fresh.

Tomatoes were chosen for the fruit vegetable. They were the Marglobe variety and were analyzed in July 1948. They were grown locally and delivered to the store in market baskets without refrigeration. The tomatoes were purchased in the morning soon after being delivered to the store.

The vegetables were all purchased at a local grocery store which did not particularly specialize in produce but maintained a good selection of vegetables. All vegetables were purchased in the morning soon after the produce had been placed on the display counters. These counters were wooden and partitioned into bins. No refrigeration was used on these counters. After purchase the vegetables were brought to the laboratory in brown paper bags.

Sampling Procedure.

Cabbage. Firm, young, green heads were selected for the early summer cabbage analyzed in June 1948. Five small and five large heads were analyzed. Firm mature heads were selected for the fall cabbage analyzed in October 1948 and again five small and five large heads were analyzed.

Any bruised or discolored outer leaves were removed from the head before cutting. No other trimming was made before the sample

was taken. For the first analysis, made on the day of purchase, a radial wedge was cut from the head. This wedge included a portion of the core, inner and outer leaves. Figure 6 illustrates how this wedge was cut. Similar wedges were cut from adjacent quadrants on succeeding days when analyses were repeated. Samples from all vegetables were cut with a stainless steel knife.

The wedges cut for each sample weighed approximately one hundred grams or slightly more, since samples for the moisture determinations were taken from the same wedge. When the small heads of cabbage were analyzed it was found that the heads were too small to provide four samples without using a cut surface. For this reason determinations were not made on the fourth day of storage. After the wedge was cut from the head the remainder was stored in the refrigerator.

A portion of the radial wedge was cut into small pieces and mixed to give a representative sample of the core, inner and outer leaves. From these representative pieces of cabbage three samples weighing two grams (± 0.5 gm.) were taken for moisture determination. These three samples were placed in aluminum drying dishes and covered immediately. Weighings were made on an analytical balance.

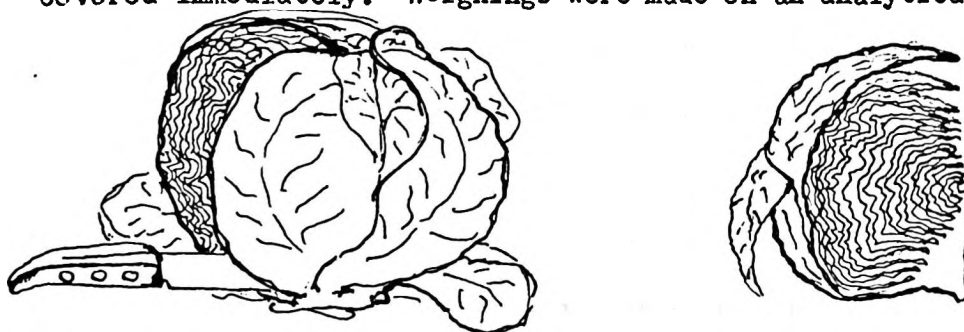


Figure 6. Sampling procedure for cabbage showing radial wedge.

Green Beans. Firm, green, medium sized beans were selected. At the laboratory the beans were wiped free of any soil with a damp cheesecloth before sampling and storage. Four series of beans were analyzed. Forty-four beans were used for each series, and a series was divided into four groups with eleven beans in each group.

Analyses were made on the day of purchase using one group of eleven beans. The remaining three groups were stored as purchased. The ends of the beans in the group analyzed were cut with a stainless steel knife and any strings removed. Nine beans were used to make the slurry. These nine beans weighed approximately one hundred grams. Each bean was cut into four pieces and dropped directly into the blender jar containing the extracting acid. The two remaining beans were finely cut, mixed to give a representative sample, and three samples each weighing two grams (± 0.5 gm.) were taken for moisture determination.

Green Peppers. Medium sized, firm green peppers were purchased. Any soil was wiped from the peppers with a damp cheesecloth before sampling and storage. Two series of peppers were analyzed. Sixteen peppers were used for each series with four peppers in each group or test sample. Analyses were made on the day of purchase using four peppers. The remaining three groups were placed in the refrigerator for storage. The four peppers in the group to be analyzed were cut into halves and the seeds and stem removed. One-half was taken from each of the four peppers and this made approximately one hundred grams for the slurry. The remaining halves of the peppers were used for moisture determinations. They were cut into small pieces with a

stainless steel knife and three samples each weighing two grams (± 0.5 gm.) were taken.

Spinach. Fresh, crisp, green spinach was selected. Two bunches or approximately two pounds were purchased for each of the four samples analyzed. Approximately sixteen plants of comparable size were selected from the two bunches. Very large or very small plants were discarded along with plants which bore wilted and bruised leaves.

Approximately twelve plants were stored in the refrigerator as purchased. Three plants were reserved for analysis on the day of purchase. Two plants with roots removed but including all leaves and stems were washed three times in tap water. After washing, the spinach was drained on a paper towel. The two plants were then wrapped loosely in cheesecloth and thoroughly shaken to remove as much moisture as possible. These two plants weighed approximately fifty grams and were used to make up the slurry. For each slurry two whole plants were used regardless of the weight so that all leaves and stems from two plants would be included.

The third plant was washed and drained as described above, finely chopped with a stainless steel knife and then mixed to give a representative sample. Three samples each weighing two grams (± 0.5 gm.) were weighed into aluminum drying dishes for moisture determinations. After two, four, and seven days of storage three plants were removed from the refrigerator and the same procedure followed.

Tomatoes. Medium sized, firm, ripe, and fully colored tomatoes were selected. Sixteen tomatoes were used in each of the

two series analyzed. The sixteen tomatoes were divided into four groups with four tomatoes in each group. The tomatoes were wiped with a damp cheesecloth before sampling and storage. The four tomatoes in each group were cut into quarters and one quarter taken from each tomato to make up the slurry. These four quarters weighed approximately one hundred grams. The remaining three groups of tomatoes were placed in the refrigerator and analyses were repeated after two, four and seven days of storage.

Each group of tomatoes was weighed on the day of purchase. A torsion balance with a sensitivity of fifteen milligrams was used for the weighings. After storage the tomatoes were allowed to come to room temperature and this required approximately twenty-five minutes. They were then weighed before sampling and any loss in weight during storage was then calculated; the weight of sample was adjusted to be comparable with the original weight. Moisture determinations were not made on tomatoes by the same method used for other vegetables. The texture of the tomatoes is different from that of the other vegetables, and it would have been difficult to obtain a representative sample from the pulp and juice to use for a moisture determination.

Method of Analysis.

Principle of analysis. The method used for this study was a macromethod for determination of reduced ascorbic acid as described by Bessey (3) and Mindlin and Butler (19). The method is based on the oxidation-reduction reaction of ascorbic acid which is quantitative. The principle is the measurement of the extent to which a sodium 2,6-dichlorobenzinoneindophenol solution is decolorized by

ascorbic acid. Ascorbic acid is readily oxidized and will react mole for mole with 2,6-dichlorobenzinoneindophenol. In the presence of an oxidizing agent which is also a colored compound, the change in color of the oxidizing agent serves as a measure of the amount of ascorbic acid in the solution being analyzed. In this method sodium 2,6-dichlorobenzinoneindophenol was used as the oxidizing agent, and the color change was measured by a photoelectric instrument (Coleman Spectrophotometer No. 11) which eliminates eye judgement of color necessary in titration methods.

The method used for determination of dehydroascrobic acid was that of Roe and Oesterling (25). The principles of the method is based upon the measurement with a photoelectric instrument of the red color produced when dehydroascorbic acid reacts with 2,4-dinitro-phenylhydrazine under carefully controlled conditions.

Preparation of slurry. Ten percent metaphosphoric acid was used as the extracting acid. This concentration was used for the slurry because it has been found to prevent destruction of ascorbic acid during the blending time and until the aliquots were weighed out and made up to volume (25).

For all vegetables except cabbage and spinach, equal weights of vegetable and ten percent metaphosphoric acid were used to make up the slurry. One hundred grams of acid and of vegetable were used. The vegetable diluted the acid to one-half its original concentration or approximately five percent metaphosphoric. The amount of acid used to make a slurry of spinach and cabbage was one and one-half times the amount of vegetable because it was found in trials that this was the amount of acid necessary to yield a homogenous slurry. Equal

amounts of acid did not sufficiently cover the spinach and cabbage in the blender jar.

For each slurry the acid was weighed into the Waring blender jar. Weighings were made on a Trip balance. The sample of vegetable was cut according to the sampling procedure described above and dropped directly into the acid in the jar and weighed. The samples were then blended for five minutes, the speed of the Waring blender being reduced to one-half with the use of a Powerstat because this slow speed helped prevent incorporation of air into the slurry.

Preparation of filtrate. An appropriate amount of slurry was weighed out and diluted to one hundred milliliters for the dehydro- and reduced ascorbic acid determinations. The appropriate amount of aliquot to be weighed out was determined by consulting food composition tables which gave the average amounts of ascorbic acid in one hundred grams of the vegetable. Bessey (3) recommends that the concentration of the filtrate for the determinations of reduced ascorbic acid be one hundred to four hundred and fifty micrograms per fifty milliliters for the dye used in this method. Roe (25) recommends twenty-five hundredths to fifteen micrograms per milliliter for the dehydroascorbic acid determinations. Two gram aliquots of the filtrate were used in the analysis of all vegetables.

Four aliquots of two grams each were weighed into one hundred milliliter beakers on a Trip balance. The aliquots were washed from the beakers into volumetric flasks 100 ml. capacity. Five percent metaphosphoric acid containing one percent thiourea was used to transfer the aliquots and make to volume for the dehydro-ascorbic acid determinations. Each of two of the remaining aliquots

were washed from the one hundred milliliter beakers into one hundred milliliter volumetric flasks with three percent metaphosphoric acid and made up to volume for the reduced ascorbic determinations. After these dilutions were thoroughly mixed they were filtered through Whatman number twelve folded filter paper. The first ten milliliters of the filtrate were discarded since there may have been some initial adsorption on the filter paper or the first few milliliters of filtrate may have been more turbid than the following portions.

Dehydroascorbic acid determinations. Four milliliters of the filtered extract made up with five percent metaphosphoric acid containing one percent thiourea were placed in each of three test tubes, one tube was held for the blank determination. To each of the two remaining tubes one milliliter of two percent 2,4 dinitrophenylhydrazine was added. The two tubes containing the dye were held at 37° C. for three hours in a constant temperature water bath. They were then cooled in ice water and the blank tube was cooled in the same way. While in the ice water, five milliliters of eighty-five percent sulfuric acid were added from a burette, one drop at a time, to each of the tubes. One full minute was used to add the five milliliters of acid so that the temperature of the solution in the test tube was not suddenly raised. This might have caused a charring of sugars or other organic substances in the solution. One milliliter of two percent 2,4-dinitrophenylhydrazine was added to the blank tubes. All tubes were thoroughly shaken in the ice bath and then removed to a rack.

After thirty minutes standing at room temperature the samples were transferred to matched cuvettes and read in a Coleman Model 11 Spectrophotometer with the wavelength set at 540 μ . The samples were read between thirty and forty-five minutes after removal from the ice bath because Roe (25) has shown the greatest intensity of color has developed after thirty minutes but starts to fade on long standing. The blank tube was used to set the galvanometer reading at one hundred. Setting the instrument to read one hundred percent transmission for the blank corrected for color in the solution other than that produced from the osazones formed during three hours in a water bath at 37° C. The blank cuvettes were replaced with cuvettes containing the solutions to be analyzed and readings made. Calculations of concentration of dehydroascorbic acid were made from a calibration curve.

Preparation of calibration curve. It was necessary to prepare a calibration curve so that the amount of dehydroascorbic acid in the vegetable extracts could be determined. This curve was prepared on semi-log paper by plotting the percent transmission against the concentrations for a series of solutions containing known amounts of ascorbic acid.

Twenty-five milligrams U.S.P. reference ascorbic acid were weighed out and made up to twenty-five milliliters with five percent metaphosphoric acid and mixed thoroughly. This gave a concentration of one milligram per one milliliter. Two drops of bromine were added to oxidize the ascorbic acid to the dehydro form; the solution shaken until yellow. The oxidized solution was decanted from excess bromine and aerated until colorless.

Ten milliliters of this solution were made up to one hundred milliliters with five percent metaphosphoric acid containing one percent thiourea. This gave a concentration of one-tenth of a milligram per one milliliter. The following dilutions were made from this concentration using five percent metaphosphoric acid containing one percent thiourea.

0.5 ml. diluted to 100 ml. concentration 0.5 mcg. per ml.
1.0 ml. diluted to 100 ml. concentration 1.0 mcg. per ml.
2.0 ml. diluted to 100 ml. concentration 2.0 mcg. per ml.
3.0 ml. diluted to 100 ml. concentration 3.0 mcg. per ml.
5.0 ml. diluted to 100 ml. concentration 5.0 mcg. per ml.
10.0 ml. diluted to 100 ml. concentration 10.0 mcg. per ml.

These dilutions were treated as described in the section for determination of dehydroascorbic acid. A calibration curve was then prepared by charting ascorbic acid concentrations and galvanometer readings on semilog paper. The values obtained and the calibration curve plotted are given in the appendix.

Reduced ascorbic acid determinations. Twenty-five milliliters of the filtered extract made up with three percent metaphosphoric acid were measured into erylenmeyer flasks. Citrate buffer was added to bring the pH to 3.5 to 3.6. The amount of buffer usually required was between seven and eight milliliters. All pH readings were made with a Beckman pH meter. These buffered extracts were then read in a Coleman Model 11 Spectrophotometer at a wavelength of 515 μ . This wavelength, 515 μ , is the wavelength at which the reaction solution, dye and buffered extract, exert its maximum optical effect. The instrument was set so that the galvanometer read one hundred for a tube containing redistilled water.

Four milliliters of buffered extract were added using a quick delivery pipette into matched cuvettes containing four milliliters of sodium 2,6-dichlorobenzidineindophenol. The solution was stirred with a glass rod and a reading was made fifteen seconds after delivery of the extract into the dye. The fifteen second reading, G_{s1} , and a reading made after thirty seconds, G_{s2} , were obtained. A small crystal of ascorbic acid was added to completely reduce the dye, and after stirring, another reading was made thirty seconds later, G_{sr} .

A buffered solution of three percent metaphosphoric acid with a pH of 3.5 to 3.6 was used to make the blank reading, G_b . The instrument was set so that the galvanometer read the same as the G_{sr} reading. Four milliliters of the buffered three percent metaphosphoric acid were added to four milliliters of the dye and stirred. After fifteen seconds a reading was made.

Calculations were made using the following formula:

$$C = K(\text{Log } G_s - \text{Log } G_b) \times \frac{\text{wt. of sample} + \text{wt. of extracting acid}}{\text{wt. of sample}}$$

$$\times \frac{100}{I} \times \frac{100}{\text{wt. of aliquot}} \times \frac{25 + \text{ml. of buffer used}}{25 (\text{ml. of filtered extract used})}$$

C = concentration of ascorbic acid in mg. per ml.

$$G_s = G_{s1} - (G_{s2} - G_{s1})$$

K = instrument constant

Determination of K . The K value which is the instrument constant depends upon the ratio between the galvanometer reading and the transmission of light in the spectrophotometer when known amounts of ascorbic acid are read. A wavelength of 515 mu. was used to determine the K value, and this setting was also used for all reduced ascorbic

acid determinations.

Twenty milligrams of ascorbic acid were dissolved in and made up to one hundred milliliters with three percent metaphosphoric acid. This gave a concentration of 0.2 mg. per ml. The following dilutions were made from the above concentration.

1 ml. diluted to 100 ml. concentration .002 mg. per ml.
 2 ml. diluted to 100 ml. concentration .004 mg. per ml.
 3 ml. diluted to 100 ml. concentration .006 mg. per ml.
 4 ml. diluted to 100 ml. concentration .008 mg. per ml.

Twenty-five milliliter aliquots were taken from each dilution and buffered with citrate buffer to a pH of 3.5-3.6. They were read in the spectrophotometer as described in the section for determination of reduced ascorbic acid.

K was calculated by the following formula:

$$K = \frac{\text{concentration (mg. per ml.)}}{\text{Log } G_s - \text{Log } G_b}$$

Sample calculation of K. Value: 1 ml. of 0.2 mg. per ml. diluted to 100 ml. gave a concentration of 0.002 mg. per ml. From this dilution 25 ml. were buffered with 7 ml. citrate buffer and gave a dilution 0.05 mg. or 50 mcg. in 32 ml. of solution or $\frac{50}{32} =$ 0.0016 mg. per ml.

$$K = \frac{.0016 \text{ mg. per ml.}}{(\text{Log } G_s - \text{Log } G_b)}$$

Moisture determinations. The percent of moisture was determined in the vegetables so that ascorbic acid values could be calculated on a dry-weight basis. When the ascorbic acid content is calculated on the dry-weight basis it is possible to determine whether there was an actual loss of ascorbic acid, or whether the difference was caused

by change in weight through gain or loss in moisture during storage. Changes in weight during storage were determined for tomatoes by weighing each sample before and after the storage period. Moisture determinations were made for cabbage, spinach, green beans, and green peppers by taking three samples each weighing two grams (± 0.5 gm.) of the plant tissue at the time of sampling for ascorbic acid analysis. These two-gram samples were weighed in aluminum drying dishes and dried in an electric oven for eight hours at 110°C . Eight hours were required before samples reached a constant weight. Samples were removed from the oven with tongs, cooled in a dessicator and weighed. All moisture determinations were made in triplicate. All weighings were made using an analytical balance.

RESULTS AND DISCUSSION

CABBAGE

Reduced Ascorbic Acid in Fresh Cabbage.

Five large and five small heads of summer cabbage were analyzed for reduced ascorbic acid and dehydroascorbic acid and all analyses were made in duplicate. The mean of these duplicates of the five large and five small heads was determined. The mean value (table 3) for five large heads was 56.4 mg. of reduced ascorbic acid per 100 gm. of cabbage, and 60.3 mg. of reduced ascorbic acid per 100 gm. for five small heads of summer cabbage. Compared with one hundred grams of fresh orange juice which contains approximately 50 mg. of reduced ascorbic acid, one hundred grams of this cabbage is a rich source of ascorbic acid.

Gould and Tressler (11) reported 32 mg. of reduced ascorbic acid per 100 gm. of fresh cabbage of the Glory variety, while Tressler, Mack, and King (30) reported 47 mg. of reduced ascorbic acid per 100 gm. of fresh Danish Ballhead cabbage. The results reported by Van Duyne (32) ranged from 42 to 47 mg. of reduced ascorbic acid per 100 gm. of fresh Racine Market cabbage. Therefore, the Jersey Wakefield variety of cabbage analyzed in this study was a richer source of reduced ascorbic acid than some other varieties reported in the literature.

Comparison of Reduced Ascorbic Acid in Large and Small Heads.

The results of analyses are summarized in table 3 and show that the large heads of cabbage had a mean value of 56.4 mg. of reduced ascorbic acid per 100 gm., and the small heads of cabbage had 60.3 mg. of reduced ascorbic acid per 100 gm. The values for the large heads

Table 3
EFFECT OF STORAGE ON THE REDUCED ASCORBIC ACID CONTENT
OF SUMMER CABBAGE

Cabbage	Reduced ascorbic acid in mg. per 100 gm.*							
	0 days		2 days		4 days		7 days	
I. Large Jersey Wakefield								
a.	61.6		53.2		49.0		48.4	
	59.0	60.3	53.7	53.4	49.1	49.0	49.1	48.8
b.	64.1		56.6		61.3		50.3	
	64.3	64.2	55.2	55.9	60.0	60.6	50.6	50.4
c.	48.0		45.1		40.4		43.1	
	47.6	47.8	43.8	44.4	39.7	40.0	42.8	43.0
d.	58.8		49.5		46.5		49.4	
	58.9	58.8	50.4	50.0	47.9	47.2	49.4	49.4
e.	50.9		50.5		43.7		42.5	
	50.7	50.8	50.3	50.4	43.7	43.7	43.0	42.8
Mean		56.4		50.8		48.1		46.9
II. Small Jersey Wakefield								
a.	60.2		45.3		-		42.0	
	60.4	60.3	44.5	44.9			42.0	42.0
b.	57.9		38.0		-		31.6	
	58.1	58.0	40.7	39.4			33.5	32.6
c.	69.9		37.5		-		37.6	
	68.9	69.4	40.1	38.8			37.4	37.5
d.	58.2		46.1		-		40.7	
	57.4	57.8	44.5	45.3			40.7	40.7
e.	55.6		46.3		-		43.3	
	56.4	56.0	47.1	46.7			41.6	42.4
Mean		60.3		43.0				39.0

*Mean of duplicate determinations for each sample are given in second column for each storage period.

of cabbage ranged from 47.8 to 64.2 mg. of reduced ascorbic acid per 100 gm., and the range for the small heads of cabbage was from 57.8 to 69.4 mg. of reduced ascorbic acid per 100 gm. These analyses show that the small heads had a slightly higher reduced ascorbic acid content than was found in the large heads of cabbage.

Effect of Storage on Reduced Ascorbic Acid.

A loss of reduced ascorbic acid in the cabbage was found after storage. In the large heads of cabbage there was a 9 percent loss after two days, a 14 percent loss after four days, and a 17 percent loss after seven days of storage. In the small heads the loss of reduced ascorbic acid was greater. After two days there was a 28 percent loss, and a 35 percent loss after seven days of storage. The greatest loss of reduced ascorbic acid occurred during the first two days of storage. The loss continued through the seventh day but was not as great as during the early part of the storage period.

Comparison of Reduced Ascorbic Acid on Fresh and Dry-Weight Basis.

Moisture determinations were made on the cabbage so that the ascorbic acid content could be calculated on the dry-weight basis. This makes it possible to determine whether there was an actual loss of ascorbic acid, or whether the difference was caused by change in weight through gain or loss of moisture in the cabbage during storage.

The mean reduced ascorbic acid value (table 4) of the large heads of cabbage calculated on the dry-weight basis was 7.6 mg. per gm. and 7.3 mg. per gm. for the small heads of cabbage. These results indicate that there was no appreciable difference in the amounts of reduced ascorbic acid in the large and small heads when compared on the dry-weight basis. After seven days storage there was a loss of

Table 4
 REDUCED ASCORBIC ACID CONTENT OF SUMMER CABBAGE
 CALCULATED ON A DRY WEIGHT BASIS

Cabbage	Reduced ascorbic acid in mg. per 100 gm.*							
	0 days		2 days		4 days		7 days	
I. Large Jersey Wakefield								
a.	9.0		7.6		7.0		7.1	
	8.7	8.8	7.7	7.6	7.0	7.0	7.2	7.2
b.	6.9		8.1		9.3		6.8	
	6.9	6.9	7.9	8.0	9.1	9.2	6.8	6.8
c.	7.3		6.7		6.2		6.7	
	7.2	7.2	6.5	6.6	6.1	6.2	6.7	6.7
d.	8.5		6.2		7.5		7.4	
	8.5	8.5	6.3	6.2	7.7	7.6	7.4	7.4
e.	6.6		5.7		5.6		5.7	
	6.6	6.6	5.7	5.7	5.6	5.6	5.8	5.8
Mean		7.6		6.8		7.1		6.8
II. Small Jersey Wakefield								
a.	9.6		6.3		-		6.5	
	9.6	9.6	6.2	6.2	-	-	6.5	6.5
b.	6.1		4.0		-		4.4	
	6.1	6.1	4.2	4.1	-	-	4.7	4.6
c.	7.8		4.1		-		4.1	
	7.6	7.7	4.4	4.2	-	-	4.1	4.1
d.	5.9		4.9		-		3.4	
	5.8	5.8	4.7	4.8	-	-	3.4	3.4
e.	7.2		6.2		-		5.0	
	7.3	7.2	6.3	6.2	-	-	4.8	4.9
Mean		7.3		5.1				4.7

*Mean of duplicate determinations for each sample are given in second column for each storage period.

10 percent of the reduced ascorbic acid in the large heads, and a loss of 35 percent in the small heads of cabbage. Again, the greatest loss of reduced ascorbic acid occurred during the first two days of storage. The loss of reduced ascorbic acid was also greater in the small heads than in the large heads of cabbage when calculated on the dry-weight basis.

Dehydroascorbic Acid in Cabbage.

The amount of dehydroascorbic acid in the fresh cabbage is shown in table 5. Dehydroascorbic acid was found to be present in both the large and small heads of cabbage. The range of dehydroascorbic acid in the large heads of cabbage was from 3.1 to 7.5 mg. per 100 gm. of cabbage giving a mean value of 5.2 mg. of dehydroascorbic acid per 100 gm. for the large heads of fresh cabbage. The range of dehydroascorbic acid in the small heads of cabbage was from 1.9 to 7.4 mg. per 100 gm. of cabbage giving a mean value of 4.9 mg. of dehydroascorbic acid per 100 gm. for the five small heads of fresh cabbage. Thus there is seen to be little difference in the amount of dehydroascorbic acid in the large and small heads of cabbage on the first day of analysis.

During seven days of storage the amount of dehydroascorbic acid increased 9 percent in the large heads of cabbage, whereas there was an increase of 28 percent in the small heads of cabbage. Calculated on the dry-weight basis there was more dehydroascorbic acid in the large heads than in the small heads of fresh cabbage. There was an increase of dehydroascorbic acid after seven days of storage in both the large and small heads. The mean values for dehydroascorbic acid show that the large heads of cabbage had increased 19 percent and the

Table 5
 DEHYDROASCORBIC ACID CONTENT
 OF FRESH AND STORED SUMMER CABBAGE

Cabbage	Dehydroascorbic acid in mg. per 100 gm.*							
	0 days		2 days		4 days		7 days	
I. Large Jersey Wakefield								
a.	6.2		6.6		5.3		5.9	
	5.9	6.0	6.6	6.6	5.3	5.3	5.3	5.6
b.	7.2		6.6		7.8		5.3	
	7.8	7.5	6.6	6.6	7.8	7.8	5.6	5.4
c.	3.1		3.1		3.1		6.2	
	3.1	3.1	3.1	3.1	3.1	3.1	6.6	6.4
d.	5.3		4.4		3.1		5.3	
	5.3	5.3	4.4	4.4	3.4	3.2	5.3	5.3
e.	3.8		4.4		5.3		5.3	
	4.4	4.1	4.7	4.6	4.4	4.8	6.2	5.8
Mean		5.2		5.1		4.8		5.7
II. Small Jersey Wakefield								
a.	3.1		4.1		-		4.4	
	3.8	3.4	3.8	4.0			4.4	4.4
b.	4.4		6.6		-		7.8	
	4.4	4.4	7.2	6.9			8.1	8.0
c.	6.9		7.8		-		7.5	
	7.5	7.2	6.9	7.4			7.8	7.6
d.	7.2		6.6		-		8.1	
	7.5	7.4	5.9	6.2			8.1	8.1
e.	2.2		2.8		-		5.9	
	1.6	1.9	3.1	3.0			6.2	6.0
Mean		4.9		5.5				6.8

*Mean of duplicate determinations for each sample are given in second column for each storage period.

small heads had increased 43 percent in dehydroascorbic acid content after seven days of storage at 40° F. It is therefore evident that the small heads of cabbage increased more in dehydroascorbic acid content during the storage period. The increase was greatest between the second and seventh day of storage.

Dehydroascorbic acid is not usually present in the growing plant, but after harvesting oxidation within the plant begins. Therefore, dehydroascorbic acid was present when this fresh cabbage was analyzed. Dehydroascorbic acid usually increases in plant tissue after cutting and storage because oxidative enzymes become more active. The results in table 3 and table 4 show that there a loss of reduced ascorbic acid during storage in both the large and small heads of cabbage. The determinations show (table 5 and table 6) that the dehydroascorbic acid content increased during storage in both the large and small heads of cabbage, but this increase of dehydroascorbic acid did not equal the decrease in reduced ascorbic acid after seven days of storage. Therefore, it is probable that some of the reduced ascorbic acid was converted beyond the dehydroascorbic acid stage to diketo-l-gulonic acid which is biologically inactive.

Comparison of Summer and Fall Cabbage.

The mean value for five large heads of fall cabbage (table 7) was 58.7 mg. of reduced ascorbic acid per 100 gm., and the mean value for the same number of small heads was 77.9 mg. When compared with the amount of reduced ascorbic acid found in summer cabbage (table 3) there is seen to be no appreciable different in the reduced ascorbic acid content of large heads of fall and summer cabbage. However, the small heads of fall cabbage were higher in reduced ascorbic acid than

Table 6

DEHYDROASCORBIC ACID CONTENT OF FRESH AND STORED SUMMER CABBAGE
CALCULATED ON A DRY WEIGHT BASIS

Cabbage	Dehydroascorbic acid in mg. per gm.*			
	0 days	2 days	4 days	7 days
I. Large Jersey Wakefield				
a.	0.912 0.868 0.890	0.943 0.943 0.943	0.757 0.757 0.757	0.868 0.779 0.824
b.	0.774 0.839 0.806	0.943 0.943 0.943	1.182 1.182 1.182	0.716 0.757 0.736
c.	0.470 0.470 0.470	0.463 0.463 0.463	0.477 0.477 0.477	0.969 1.031 1.000
d.	0.768 0.768 0.768	0.550 0.550 0.550	0.500 0.548 0.524	0.791 0.791 0.791
e.	0.494 0.571 0.532	0.534 0.500 0.517	0.679 0.564 0.622	0.716 0.838 0.777
Mean	0.693	0.683	0.712	0.826
II. Small Jersey Wakefield				
a.	0.492 0.603 0.548	0.569 0.528 0.548	-	0.677 0.677 0.677
b.	0.463 0.463 0.463	0.688 0.750 0.719	-	1.088 1.141 1.120
c.	0.767 0.833 0.800	0.857 0.758 0.808	-	0.824 0.857 0.840
d.	0.727 0.758 0.742	0.702 0.628 0.665	-	0.686 0.686 0.686
e.	0.286 0.208 0.247	0.373 0.413 0.393	-	0.678 0.713 0.696
Mean	0.560	0.627		0.804

*Mean of duplicate determinations for each sample are given in second column for each storage period.

Table 7
EFFECT OF STORAGE ON THE REDUCED ASCORBIC ACID CONTENT
OF FALL CABBAGE

Cabbage	Reduced ascorbic acid in mg. per 100 gm.*							
	0 days		2 days		4 days		7 days	
I. Large Flat Dutch								
a.	44.5		45.6		47.1		47.4	
	44.2	44.4	46.0	45.8	47.2	47.2	45.2	46.3
b.	53.4		51.1		48.0		52.8	
	48.0	50.7	51.5	51.3	48.2	48.1	53.2	53.0
c.	71.7		78.0		71.1		70.2	
	72.6	72.2	77.1	77.6	72.0	71.6	70.8	70.5
d.	54.2		52.4		50.0		42.9	
	53.9	54.0	49.5	51.0	51.1	50.8	47.9	45.4
e.	71.6		52.1		54.3		53.7	
	72.5	72.0	53.1	52.6	55.2	54.8	54.4	54.0
Mean		58.7		55.7		54.5		53.8
II. Small Jersey Wakefield								
a.	75.5		73.9		-		70.2	
	70.1	72.8	71.2	72.6			70.0	70.1
b.	72.0		80.0		-		70.4	
	70.9	71.4	78.8	79.4			71.9	71.2
c.	92.8		81.6		-		63.5	
	88.0	90.4	84.8	83.2			63.9	63.7
d.	95.4		95.2		-		69.7	
	96.4	95.9	94.6	94.9			71.5	70.6
e.	59.2		59.1		-		56.8	
	58.4	58.8	56.4	57.8			54.7	55.8
Mean		77.9		77.6				66.3

*Mean of duplicate determinations for each sample are given in second column for each storage period.

the small heads analyzed in the summer. This increase of reduced ascorbic acid in the small heads of fall cabbage may have been caused by the fact that the summer cabbage was grown in South Carolina, and the fall cabbage was grown in Wisconsin. Soil and climate are factors which affect the rate of growth in plants.

After storage for seven days there was a loss of reduced ascorbic acid in the heads of fall cabbage. The large heads had lost 8 percent and the small heads, 15 percent of their original reduced ascorbic acid content at the end of seven days of storage. Calculated on the dry-weight basis (table 8) the amount of reduced ascorbic acid was 6.7 mg. per gm. in the large heads of fall cabbage, and 8.1 mg. of reduced ascorbic acid per gm. in the small heads of fall cabbage. There was no loss in reduced ascorbic acid content after seven days storage in the large heads of fall cabbage calculated on the dry-weight basis, but a 17 percent loss occurred in the small heads. The greatest loss of reduced ascorbic acid occurred in the small heads between the second and seventh day of storage.

The values for the dehydroascorbic acid content of the large and small heads of fall cabbage are summarized in table 9. The mean value found for the five large heads was 4.9 mg. of dehydroascorbic acid per 100 gms. of cabbage. When compared with the amount of dehydroascorbic acid found in the large heads of summer cabbage (table 5) there is seen to be no appreciable difference in the large heads of fall and summer cabbage. The mean value for the small heads of fall cabbage was found to be 7.5 mg. of dehydroascorbic acid per 100 gm. of cabbage which is greater than the amount found in the small heads of summer

Table 8
 REDUCED ASCORBIC ACID CONTENT OF FALL CABBAGE
 CALCULATED ON A DRY WEIGHT BASIS

Cabbage	Reduced ascorbic acid in mg. per gm.*							
	0 days		2 days		4 days		7 days	
I. Large Flat Dutch								
a.	5.8		5.6		5.4		5.9	
	5.8	5.8	5.6	5.6	5.4	5.4	5.6	5.8
b.	6.0		5.6		5.4		5.5	
	5.4	5.7	5.6	5.6	5.4	5.4	5.6	5.6
c.	7.3		7.8		7.1		7.5	
	7.4	7.4	7.7	7.8	7.2	7.2	7.6	7.6
d.	5.9		5.6		5.4		4.3	
	5.9	5.9	5.3	5.4	5.6	5.5	4.8	4.6
e.	8.5		6.1		5.9		5.9	
	8.6	8.6	6.2	6.2	6.0	6.0	6.0	6.0
Mean		6.7		6.1		5.9		5.9
II. Small Jersey Wakefield								
a.	8.6		8.1		-		6.9	
	8.0	8.3	7.8	8.0			6.9	6.9
b.	8.0		8.3		-		7.5	
	8.8	8.4	8.2	8.2			7.7	7.6
c.	8.6		8.2		-		6.0	
	8.2	8.4	8.5	8.4			6.0	6.0
d.	9.6		9.7		-		7.1	
	9.7	9.6	9.6	9.6			7.2	7.2
e.	6.1		6.8		-		5.8	
	6.0	6.0	6.5	6.6			5.6	5.7
Mean		8.1		8.2				6.7

*Mean of duplicate determinations for each sample are given in second column for each storage period.

Table 9
 DEHYDROASCORBIC ACID CONTENT
 OF FRESH AND STORED FALL CABBAGE

Cabbage	Dehydroascorbic acid in mg. per 100 gm.*							
	0 days		2 days		4 days		7 days	
I. Large Flat Dutch								
a.	3.4		6.0		2.6		5.0	
	5.0	4.2	5.6	5.6	3.1	2.8	5.4	5.2
b.	1.6		6.2		1.8		5.6	
	3.1	2.4	5.6	5.9	2.5	2.2	5.2	5.4
c.	7.0		3.1		5.0		5.0	
	7.4	7.2	3.4	3.2	5.6	5.3	5.2	5.1
d.	6.4		2.5		4.6		4.6	
	6.2	6.3	2.0	2.2	4.3	4.4	4.4	4.5
e.	4.8		2.8		5.3		4.3	
	4.3	4.6	3.1	3.0	4.8	5.0	3.1	3.7
Mean		4.9		4.0		3.9		4.8
II. Small Jersey Wakefield								
a.	9.2		5.8		-		6.2	
	8.1	8.6	6.2	6.0	-	-	6.4	6.3
b.	7.4		6.5		-		5.3	
	8.0	7.7	6.8	6.6	-	-	5.6	5.4
c.	9.0		3.0		-		6.8	
	8.7	8.8	3.1	3.0	-	-	6.4	6.6
d.	10.0		8.1		-		5.0	
	8.7	9.4	8.0	8.0	-	-	6.2	5.6
e.	3.1		4.3		-		5.6	
	3.1	3.1	3.7	4.0	-	-	5.0	5.3
Mean		7.5		5.5				5.8

*Mean of duplicate determinations for each sample are given in second column for each storage period.

Table 10

DEHYDROASCORBIC ACID CONTENT OF FRESH AND STORED FALL CABBAGE
CALCULATED ON A DRY WEIGHT BASIS

Cabbage	Dehydroascorbic acid in mg. per gm.*			
	0 days	2 days	4 days	7 days
I. Large Flat Dutch				
a.	0.447 0.657 0.552	0.740 0.691 0.716	0.302 0.360 0.331	0.625 0.675 0.650
b.	0.181 0.352 0.267	0.681 0.615 0.648	0.204 0.284 0.244	0.589 0.547 0.568
c.	0.714 0.755 0.734	0.310 0.340 0.325	0.500 0.560 0.530	0.538 0.559 0.548
d.	0.703 0.681 0.692	0.269 0.215 0.242	0.505 0.472 0.488	0.465 0.444 0.454
e.	0.571 0.512 0.542	0.329 0.365 0.347	0.576 0.522 0.549	0.478 0.344 0.411
Mean	0.557	0.455	0.428	0.526
II. Small				
a.	1.057 0.931 0.994	0.637 0.681 0.659	-	0.614 0.634 0.624
b.	0.882 0.889 0.856	0.677 0.708 0.692	-	0.570 0.602 0.586
c.	0.841 0.813 0.827	0.303 0.313 0.308	-	0.648 0.610 0.629
d.	1.010 0.879 0.944	0.826 0.816 0.821	-	0.510 0.633 0.572
e.	0.323 0.323 0.323	0.500 0.430 0.465	-	0.577 0.515 0.546
Mean	0.789	0.589		0.591

*Mean of duplicate determinations for each sample are given in second column for each storage period.

cabbage.

After seven days storage there was no increase in dehydroascorbic acid in the five large heads of fall cabbage, but there was a 22 percent loss of dehydroascorbic acid in the small heads of fall cabbage. Values calculated on a dry-weight basis also show a loss of dehydroascorbic acid in the small heads of fall cabbage, but there was no gain or loss of dehydroascorbic acid shown in the large heads of fall cabbage.

Discussion Regarding Loss of Ascorbic Acid during Storage.

A total of twenty heads of cabbage were analyzed, and the results show that this vegetable is a good source of ascorbic acid. Ten heads of cabbage were analyzed in the summer, and ten heads were analyzed in the fall. The summer cabbage was grown in South Carolina, and the fall cabbage in Wisconsin, and both had been harvested several days before analyses were made. The mean values show that the small heads of both summer and fall cabbage contained more reduced ascorbic acid than the large heads. This may have been caused by a greater enzyme activity in the large heads after the cabbage was harvested. There was a small loss of reduced ascorbic acid after seven days storage in all the heads analyzed, and this loss was greatest during the first two days of storage. The loss of reduced ascorbic acid during the first two days of storage may have been caused by an increase of enzyme activity after the initial cutting of the head. Apparently the enzyme activity was less at the end of two days but continued during the seven days of storage.

Dehydroascorbic acid was found to be present in all the heads of cabbage when first analyzed, but after seven days of storage the dehydroascorbic acid content had not appreciably increased. There was actually a small loss of total ascorbic acid during the storage period since the increase of dehydroascorbic acid did not equal the decrease of reduced ascorbic acid. Calculations for reduced and dehydroascorbic acid were made on a dry-weight basis, but there was very little change in the values obtained since the cabbage showed no loss in moisture during storage.

SPINACH

Comparison of Reduced and Dehydroascorbic Acid in Fresh and Stored Spinach.

The mean value for reduced ascorbic acid in fresh spinach was 38.6 mg. per 100 gm. (table 11). Four samples of Aragon spinach were analyzed and the range in the reduced ascorbic acid values of the four samples showed wide variation. The values ranged from 14.3 to 67.4 mg. of reduced ascorbic acid per 100 gm. of spinach. This variation may have been due to differences in the samples taken even though two whole plants including stems and all outer leaves were used for each determination.

At the end of the seven day storage period the spinach had lost 27 percent of the original reduced ascorbic acid. No single sample showed a consistent loss, but the mean value for all samples showed a consistent trend with the greatest loss of reduced ascorbic acid occurring during the first two days of storage. Calculated on the dry-weight basis the amount of reduced ascorbic acid in the spinach showed less variation than on the fresh weight. This is because the

Table 11

REDUCED ASCORBIC ACID CONTENT OF FRESH AND STORED SPINACH

(Fresh calculated in mg. per 100 gm.; dry weight calculated in mg. per gm.)

Sample	0 days		2 days		4 days		7 days	
	fresh	dry wt.	fresh	dry wt.	fresh	dry wt.	fresh	dry wt.
a.	26.5	3.2	8.3	1.1	15.0	1.7	7.5	0.9
	23.3	3.0	7.8	1.0	15.3	1.7	7.7	0.9
Mean	24.4	3.1	8.0	1.0	15.2	1.7	7.6	0.9
b.	66.5	6.8	63.3	6.1	48.8	5.1	57.4	5.4
	68.4	7.0	67.1	6.0	48.2	5.0	57.3	5.4
Mean	67.4	6.9	67.7	6.0	48.5	5.1	57.4	5.4
c.	14.4	1.7	9.7	1.3	8.1	1.0	6.9	0.7
	14.3	1.7	10.3	1.4	8.5	1.1	8.4	0.9
Mean	14.4	1.7	10.0	1.4	8.3	1.0	7.6	0.8
d.	47.9	5.0	41.6	4.0	46.7	4.2	42.9	4.2
	48.7	5.1	49.0	4.7	47.3	4.2	35.9	3.6
Mean	48.3	5.0	45.3	4.4	47.0	4.2	39.4	3.8
Mean of 4 samples	38.6	4.2	32.8	3.2	29.8	3.0	28.0	2.8

Table 12

DEHYDROASCORBIC ACID CONTENT OF FRESH AND STORED SPINACH

(Fresh calculated in mg. per 100 gm.; dry weight calculated in mg. per gm.)

Sample	0 days		2 days		4 days		7 days	
	fresh	dry wt.	fresh	dry wt.	fresh	dry wt.	fresh	dry wt.
a.	5.3	0.688	1.6	0.219	4.6	0.535	2.9	0.358
	3.4	0.442	3.2	0.438	2.8	0.326	2.3	0.284
Mean	4.4	0.565	2.4	0.328	3.7	0.430	2.6	0.321
b.	3.2	0.330	5.6	0.504	4.5	0.474	4.2	0.396
	3.4	0.351	6.5	0.586	4.2	0.442	4.8	0.453
Mean	3.3	0.340	6.0	0.545	4.4	0.458	4.5	0.424
c.	1.6	0.192	1.6	0.225	2.5	0.333	2.4	0.273
	2.6	0.321	2.8	0.394	2.5	0.333	3.0	0.341
Mean	2.1	0.260	2.2	0.310	2.5	0.333	2.7	0.307
d.	1.5	0.160	3.1	0.298	5.0	0.450	5.0	0.495
	1.5	0.160	2.5	0.240	2.5	0.225	4.3	0.426
Mean	1.5	0.160	2.8	0.269	3.8	0.338	4.6	0.460
Mean of four samples	2.8	0.331	3.4	0.363	3.6	0.390	3.6	0.378

spinach lost weight in the refrigerator and adjustments needed to be made for this weight change.

Dehydroascorbic acid was found to be present in the four samples of spinach analyzed. The mean value for fresh spinach (table 12) was 2.8 mg. of dehydroascorbic acid per 100 gm. of spinach, and after seven days storage the amount had increased to 3.6 mg. per 100 gm. of spinach.

The loss of reduced ascorbic acid in these samples of spinach was no greater after storage than other investigators have reported. These samples of spinach were found to be a good source of ascorbic acid. The loss of reduced ascorbic acid was greater in the spinach than in the cabbage, and this might be expected in the spinach because the leaves of the plant are very active. Photosynthesis occurs in the leaves and more enzyme activity would be expected in the thin spinach leaves than in a cabbage head which is more compact and has thicker leaves.

GREEN BEANS

Effect of Storage on the Reduced and Dehydroascrobic Acid Content of Green Beans.

The mean value for four samples of green beans was 11.6 mg. of reduced ascorbic acid per 100 gm.; the range was 5.4 to 16.5 mg. of reduced ascorbic acid per 100 gm. of beans (table 13). This amount of reduced ascorbic acid found in these green beans shows that they were not a good source of vitamin C. The loss of reduced ascorbic acid after seven days was 27 percent, but the mean value did not show a consistent loss at any certain time of the storage period. Spinach

Table 13

REDUCED AND DEHYDROASCORBIC ACID CONTENT OF FRESH AND STORED GREEN BEANS

(Mg. per 100 gms.)

Sample	0 days		2 days		4 days		7 days	
	Reduced	Dehydro	Reduced	Dehydro	Reduced	Dehydro	Reduced	Dehydro
a.	17.1	0.8	10.6	2.1	8.6	2.8	8.8	2.7
	15.8	0.7	11.3	1.3	9.8	2.2	9.2	2.2
Mean	16.5	0.8	11.0	1.7	9.2	2.5	9.0	2.4
b.	12.7	1.3	7.8	2.1	9.1	2.3	8.5	2.3
	13.1	0.8	8.6	1.3	8.1	2.3	9.0	2.4
Mean	12.9	1.0	8.2	1.7	8.6	2.3	8.8	2.3
c.	5.6	1.2	9.6	1.2	9.5	2.6	5.7	2.2
	5.2	0.7	9.1	1.2	9.5	2.4	5.7	3.8
Mean	5.4	1.0	9.4	1.2	9.5	2.5	5.7	3.0
d.	11.9	0.8	11.9	2.0	6.4	2.1	9.7	4.6
	10.8	1.3	11.4	1.4	6.6	2.4	10.3	2.9
Mean	11.4	1.0	11.6	1.7	6.5	2.2	10.0	3.8
Mean of four samples	11.6	1.0	10.0	1.6	8.4	2.4	8.4	2.9

Table 14

EFFECT OF STORAGE ON THE REDUCED AND DEHYDROASCORBIC ACID CONTENT OF GREEN BEANS
CALCULATED ON A DRY WEIGHT BASIS
(Mg. per gm.)

Sample	0 days		2 days		4 days		7 days	
	Reduced	Dehydro	Reduced	Dehydro	Reduced	Dehydro	Reduced	Dehydro
a.	2.2	0.104	1.1	0.236	1.0	0.350	0.8	0.267
	2.0	0.091	1.2	0.146	1.2	0.275	0.9	0.218
Mean	2.1	0.098	1.2	0.191	1.1	0.312	0.8	0.242
b.	1.7	0.181	0.8	0.223	1.0	0.258	0.7	0.204
	1.8	0.111	0.9	0.138	0.9	0.258	0.7	0.212
Mean	1.8	0.146	0.8	0.180	0.9	0.258	0.7	0.208
c.	0.8	0.176	1.1	0.146	0.9	0.257	0.4	0.185
	0.7	0.103	1.1	0.146	0.9	0.238	0.4	0.319
Mean	0.8	0.140	1.1	0.146	0.9	0.248	0.4	0.252
d.	1.3	0.092	1.3	0.233	0.6	0.221	0.8	0.407
	1.2	0.149	1.3	0.163	0.6	0.253	0.9	0.257
Mean	1.2	0.120	1.3	0.198	0.6	0.237	0.8	0.332
Mean of four samples	1.5	0.126	1.1	0.179	0.9	0.264	0.7	0.258

and cabbage lost more of the reduced ascorbic acid during the first two days of storage than did the green beans.

Dehydroascorbic acid was present in all four samples of green beans. The green beans showed a much greater increase of dehydroascorbic acid after seven days storage than was shown in the cabbage and spinach. There was over a 100 percent increase of dehydroascorbic acid in the green beans after seven days both when calculations were made for the fresh beans and on a dry-weight basis. This increase of dehydroascorbic acid almost equals the decrease of reduced ascorbic acid in the green beans after seven days of storage thus making the loss of total ascorbic acid very small. The loss of the vitamin was not expected to be great since the bean is a pod type of vegetable containing seed. This edible part of the plant is very different in structure from the leafy type of vegetable and is not expected to be as active. Leaves contain more chlorophyll than the pod, therefore metabolic activity is more rapid.

The ascorbic acid content found in these beans before and after storage is comparable to the values reported in the literature by other investigators. Feener (9) reported that the greatest loss of ascorbic acid occurred in beans during the first twenty-four hours of storage in a refrigerator at 1 to 3° C. The beans analyzed in this study showed a loss in weight after seven days storage, and on the last day of analysis the beans were no longer crisp but had become limp.

GREEN PEPPERS

Comparison of Reduced and Dehydroascorbic Acid in Fresh and Stored Green Peppers.

The data in table 15 show that the mean value for reduced ascorbic

Table 15
 EFFECT OF STORAGE ON THE REDUCED ASCORBIC ACID
 CONTENT OF GREEN PEPPERS
 (mg. per 100 grams)

Sample	0 days	2 days	4 days	7 days
a.	63.3	49.6	58.2	53.6
	63.3	52.1	57.5	56.3
b.	77.2	72.8	70.5	67.4
	76.9	73.7	71.2	67.0
Mean	70.2	62.0	64.3	61.1

Table 16
 EFFECT OF STORAGE ON THE REDUCED ASCORBIC ACID CONTENT
 OF GREEN PEPPERS WHEN CALCULATED ON A DRY WEIGHT BASIS
 (mg. per gm.)

Sample	0 days	2 days	4 days	7 days
a.	9.4	9.2	9.7	8.2
	9.4	9.6	9.9	8.7
b.	12.4	9.1	11.2	10.4
	12.4	9.2	11.3	10.3
Mean	10.9	9.3	10.5	9.4

Table 17

DEHYDROASCORBIC ACID CONTENT OF FRESH AND STORED GREEN PEPPERS
(mg. per 100 gms.)

Sample	0 days	2 days	4 days	7 days
Bell				
a.	4.2	4.5	5.2	5.2
	4.8	4.2	4.8	5.2
b.	2.5	3.0	3.5	3.0
	3.0	2.9	2.9	3.8
Mean	3.6	3.7	4.1	4.3

Table 18

DEHYDROASCORBIC ACID CONTENT OF FRESH AND STORED GREEN PEPPERS
WHEN CALCULATED ON A DRY WEIGHT BASIS
(mg. per gm.)

Sample	0 days	2 days	4 days	7 days
Bell				
a.	0.627	0.889	0.881	0.800
	0.716	0.778	0.814	0.800
b.	0.403	0.375	0.556	0.462
	0.484	0.362	0.460	0.585
Mean	0.558	0.601	0.678	0.662

acid in fresh green peppers was 70.2 mg. per 100 gm. Green peppers thus are seen to be an excellent source of ascorbic acid. After seven days of storage there was an 11 percent loss of reduced ascorbic acid and a 13 percent loss when calculated on a dry-weight basis.

Dehydroascorbic acid was present in the two samples of green peppers. The mean value was 3.6 mg. of dehydroascorbic acid per 100 mg. of pepper, and after seven days of storage there was an increase of 16 percent. The loss in reduced ascorbic acid was greatest during the first two days of storage, but the greatest increase in dehydroascorbic acid occurred between the second and fourth day of storage.

Green peppers showed a better retention of the vitamin than did cabbage, spinach, and green beans. After seven days of storage these peppers were still moist and crisp.

TOMATOES

The Reduced and Dehydroascorbic Acid Content of Tomatoes Before and After Storage.

The mean value of 35.8 mg. of reduced ascorbic acid per 100 gm. of tomatoes (table 19) indicates that fresh tomatoes are comparable in vitamin C content to spinach, are higher than green beans, and lower than cabbage and green peppers. After seven days storage there was a loss of 10 percent of the reduced ascorbic acid content, and the greatest loss apparently occurred between the second and fourth day of storage. Calculations made with corrections for loss in weight showed little difference in values from those calculated on the fresh tomatoes. Moisture determinations were not made, but changes in weight were determined by weighing each group of tomatoes on the day of purchase and again on the day of analysis.

Table 19
 REDUCED ASCORBIC ACID IN FRESH AND STORED TOMATOES
 (mg. per 100 gm.)

Sample	0 days	2 days	4 days	7 days
a.	32.6	28.9	24.5	31.8
	33.1	30.5	24.9	31.9
b.	38.7	34.2	29.5	28.7
	38.8	34.1	29.1	28.7
Mean	35.8	32.0	27.0	30.2

Table 20
 REDUCED ASCORBIC ACID IN FRESH AND STORED TOMATOES
 WITH CORRECTIONS FOR LOSS IN WEIGHT
 (mg. per 100 gm.)

Sample	0 days	2 days	4 days	7 days
a.	32.6	28.8	24.5	31.7
	33.1	30.5	24.9	31.8
b.	38.7	34.1	29.3	28.5
	38.8	34.1	29.0	28.5
Mean	35.8	31.8	27.0	30.2

Table 21
 DEHYDROASCORBIC ACID IN FRESH AND STORED TOMATOES
 (mg. per 100 gm.)

Sample	0 days	2 days	4 days	7 days
a.	3.0	4.2	4.8	5.2
	2.5	4.8	5.8	4.8
b.	2.5	3.0	6.2	5.2
	3.0	3.0	5.2	5.8
Mean	2.8	3.8	5.5	5.2

Table 22
 DEHYDROASCORBIC ACID IN FRESH AND STORED TOMATOES
 WITH CORRECTIONS FOR LOSS IN WEIGHT
 (mg. per 100 gm.)

Sample	0 days	2 days	4 days	7 days
a.	3.0	4.2	4.7	5.2
	2.5	4.7	5.7	4.7
b.	2.5	3.0	6.2	5.2
	3.0	3.0	5.2	5.7
Mean	2.8	3.7	5.4	5.2

Data in table 21 show that the mean value was 2.8 mg. of dehydroascorbic acid per 100 gm. of tomatoes. There was an increase of dehydroascorbic acid of almost 100 percent after seven days of storage. The greatest increase of dehydroascorbic acid occurred between the second and fourth day of storage and corresponded to the period of greatest decrease of reduced ascorbic acid. These results show that the change from the reduced to the dehydro form of ascorbic acid occurred at the same time during the storage period. This was not the case for the other vegetables analyzed.

The loss of total ascorbic acid was almost negligible in these tomatoes. The gain in dehydroascorbic acid almost equalled the loss in reduced ascorbic acid. The low pH of the juice in the tomato is believed to be a protection against oxidation within the fruit. The tomatoes are protected by a skin, therefore little loss of ascorbic acid was expected. Brown and Moser (6) reported no appreciable loss of ascorbic acid in tomatoes after one week of storage at 44° F.

GENERAL DISCUSSION

A loss of ascorbic acid was expected in the vegetables because of oxidative enzymes naturally present in plant tissues. A loss did occur in the vegetables after seven days of storage at 40° F. in a household refrigerator. The greatest loss of ascorbic acid seemed to occur during the first two days of storage. This loss during the early part of the storage period may have been caused by an increase in enzyme activity after the initial sampling. The enzyme activity was apparently less at the end of two days but continued throughout the storage period.

Some of the analyses show wide variation between reduced and dehydroascorbic acid content in the samples. When calculated on a dry-weight basis some of these variations were not so large. Many investigators feel that data are more comparable when reported on a dry-weight basis. In this report, the ascorbic acid content has been given in both ways, because the determinations calculated on the dry-weight basis eliminate the problem of change in moisture content of foods during storage.

Dehydroascorbic acid was found to be present in all of the vegetables analyzed. The increase of dehydroascorbic acid was not great during the seven days storage period for cabbage, spinach, and green peppers, but tomatoes and green beans showed a 100 percent increase after seven days storage.

Because respiratory enzymes are present in all living cells, all plant foodstuffs lose their vitamin C content at an appreciable rate when stored. Such vegetables as potatoes, cabbage, parsnips, rutabagas, carrots, and onions have a low rate of respiration and evaporation since they possess large bulk. These vegetables commonly lose about one-half of their original ascorbic acid content during a season's storage of several weeks (9). Vegetables such as fresh spinach, lettuce, green peas and beans lose their ascorbic acid value rapidly at room temperature and fairly rapidly even under refrigerated storage.

Since temperature is an important factor in the preservation of ascorbic acid in plant foods, and since these temperature effects can be correlated with enzyme activity (9), it has been suggested that the preservation of vitamin C is primarily an enzyme problem.

There have been reports (10) that enzyme activity doubles with each

10° C. rise in temperature until the optimum is reached, and that enzyme activity can be correspondingly inhibited by lowering the temperature. Therefore refrigeration can play an important role in preserving the vitamin C value of foods. However it must be recognized that some enzyme activity continues even at freezing temperature and deterioration may slowly take place even at this temperature.

In the case of vegetables and fruits Stone and Zilva (29) have found that in some plant tissue there exists certain enzymes which are destructive to vitamin C, while in other vegetables, there is little or none of these enzymes present. This may account for the differences in this rate of destruction of ascorbic acid as shown by some of the vegetables during storage. It is therefore concluded that the rate of destruction of the vitamin depends upon the concentration of oxidative enzymes within the plant tissue as well as upon the temperature affecting their activity.

Green beans were the only vegetable that lost weight during storage; the other four vegetables remained at approximately their original weight. These findings are evidence of decreased evaporation from the vegetables kept inside of the storage chamber in the refrigerator, and indicate that the glass shelves and doors of the chamber retarded air circulation.

SUMMARY

The ascorbic acid content of vegetables stored in a household refrigerator was determined. The vegetables used were cabbage, spinach, green beans, green peppers, and tomatoes and these were chosen because they are widely used throughout the country. These vegetables are typical of different parts of the plant which are served as vegetables and included head, leaf, pod, and fruit part of the plant.

The vegetables were stored in a vegetable pan inside the moist chamber of a refrigerator which had been especially designed by the manufacturer for vegetable storage. The temperature of the moist chamber was 38 to 42° F.

The storage periods were two days, four days, and seven days. These were believed to be representative of the average length of time a homemaker might use for vegetable storage.

The amount of reduced and dehydroascorbic acid was determined in duplicates on each vegetable sample. Moisture determinations were made in triplicate on each sample. Calculations of vitamin content were also made on the dry-weight basis. Reduced ascorbic acid was determined by the macromethod as described by Bessey, and by Mindlin and Butler. Dehydroascorbic acid was determined by the method of Roe and Oesterling for the direct determination of dehydroascorbic acid.

Samples of both summer and fall cabbage were analyzed and were found to be comparable in ascorbic acid content. The mean value for five large heads was 56.4 mg. of reduced ascorbic acid per 100 gm. of cabbage, and 60.3 mg. per 100 gm. for five small heads of summer cabbage. Size of head made a difference; the small heads of both summer and fall cabbage contained more ascorbic acid than the large

heads for both seasons. There was a small loss of ascorbic acid when the cabbage was stored seven days. This loss was greatest during the first two days of storage. Dehydroascorbic acid was present in all heads when first analyzed and the mean value for the large heads of summer cabbage was 5.2 mg. per 100 gm, and for the small heads was 4.9 mg. per 100 gm. of cabbage. The large heads of fall and summer cabbage had comparable amounts of dehydroascorbic acid, but the small heads of fall cabbage contained 7.5 mg. of dehydroascorbic acid per 100 gm. of cabbage compared with 4.9 mg. per 100 gm. of summer cabbage. This form of the vitamin did not increase appreciably during the storage period.

Four samples of spinach were analyzed and the reduced ascorbic acid content showed wide variation. The mean value for reduced ascorbic acid in fresh spinach was 38.6 mg. per 100 gm. There was a 27 percent loss at the end of seven days storage and the greatest loss occurred during the first two days of storage. The dehydroascorbic acid content of spinach was 2.8 mg. per 100 gm. of spinach, and there was only a slight increase during storage.

Green beans contained 11.6 mg. of reduced ascorbic acid per 100 gm. They lost little of their total ascorbic acid during storage because the increase in dehydroascorbic acid almost equalled the decrease in reduced ascorbic acid. However, the beans showed a greater loss in weight during storage than was shown in the other vegetables.

Two samples of green peppers were analyzed and were found to be the richest source of all the vegetables analyzed for vitamin C; there was 70.2 mg. reduced ascorbic acid per 100 gm. of peppers.

Dehydroascorbic acid was present in small amounts; 3.6 mg. per 100 gm. The loss of total ascorbic acid was small after seven days of storage, and the greatest loss occurred during the first two days of storage.

Two samples of tomatoes were analyzed and contained 35.8 mg. of reduced ascorbic acid per 100 gm. of tomatoes. After seven days storage there was a loss of 10 percent of the reduced ascorbic acid, but the dehydroascorbic acid had increased almost 100 percent. The loss of reduced ascorbic acid and the gain of dehydroascorbic acid occurred between the second and fourth day of storage.

There was no appreciable loss of ascorbic acid in green beans, green peppers, and tomatoes, and only a slight loss in spinach and cabbage after seven days of storage.

All the vegetables except green beans were found to be good sources of vitamin C, and the analyses showed results comparable to those reported in the literature for fresh and stored vegetables.

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Appendix A

Reagents used and method for preparation

6% metaphosphoric acid. 60 grams glacial HPO_3 were dissolved in redistilled water and made up to 1000 ml. It was filtered, stored in the refrigerator, and discarded after fifteen days.

3% metaphosphoric acid. This was prepared from 6% HPO_3 as needed by diluting with redistilled water.

Citrate buffer. 21.0 grams of citric acid were dissolved in 200 ml. of 1.0 N NaOH and diluted to 250 ml. with carbonate free redistilled water.

5% metaphosphoric acid containing 1% thiourea. 50 grams glacial HPO_3 and 10 grams thiourea were dissolved in redistilled water and made up to 1000 ml. It was filtered, stored in the refrigerator, and discarded after fifteen days.

10% metaphosphoric acid. 100 grams glacial HPO_3 were dissolved in redistilled water and made up to 1000 ml. It was filtered, stored in the refrigerator, and discarded after fifteen days.

2,6-dichlorobenzinoneindophenol. 8 mg. 2,6-dichlorobenzinoneindophenol (Eastman Kodak preparation) were dissolved in warm redistilled water, cooled, and made to 500 ml. with redistilled water. It was filtered, stored in the refrigerator in a dark brown bottle, and discarded after ten days.

2% 2,4-dinitrophenylhydrazine. 2 grams of 2,4-dinitrophenylhydrazine (Eastman Kodak preparation) were dissolved in 100 ml. of approximately 9 N H_2SO_4 and filtered.

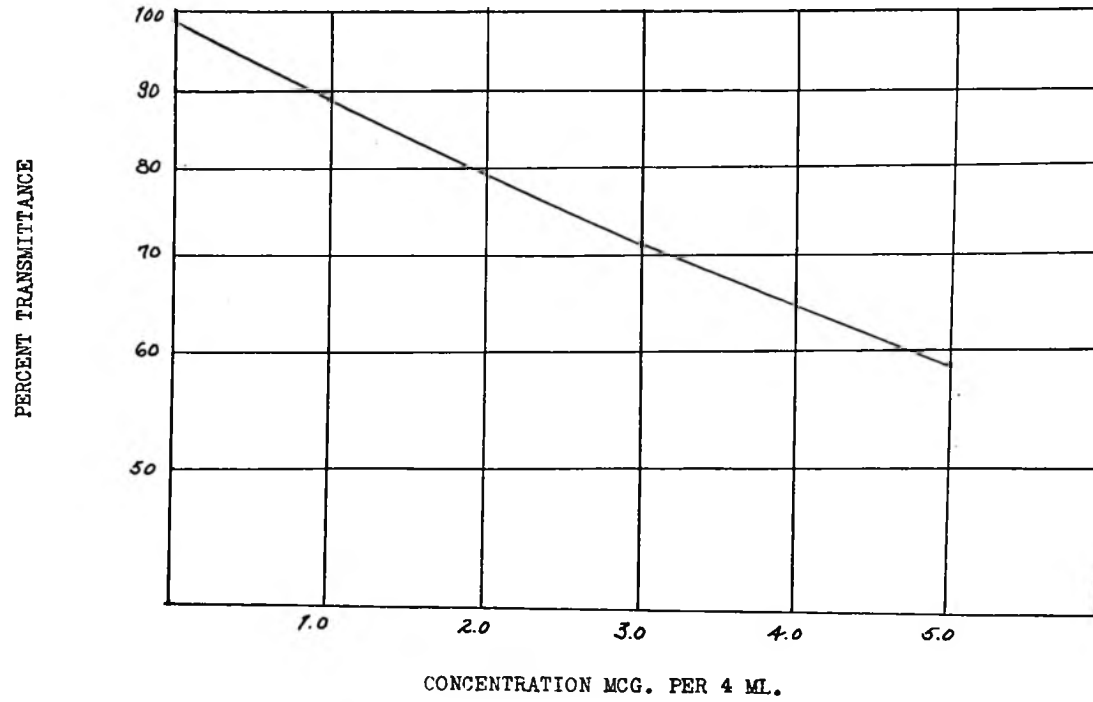
Approximately 9 N H_2SO_4 . One volume of concentrated sulfuric acid (sp. gr. 1.84) was added to three volumes of redistilled water.

85% H_2SO_4 . 900 ml. concentrated sulfuric acid (sp. gr. 1.84) were added to 100 ml. redistilled water.

Stock standard ascorbic acid solution. 25 mg. ascorbic acid were dissolved in 5% HPO_3 and made up to 25 ml. This was mixed thoroughly.

Appendix B

Calibration Curve Used for Dehydroascorbic Determinations



Appendix C

Equipment

Item		Description	Size
Balances	1	Torsion, chemical	Capacity 500 grams Sensitivity 15 mgs.
	1	Harvard trip, double agate bearings	Sensitivity .1 gram
	1	Becker analytical Chainomatic	Capacity 100 grams Sensitivity .1 mg.
Battery	1	Willard storage	4 cell, 8 volt
Battery Charger	1	100 volts, 75 watts	
Beakers	2	Pyrex	10 ml.
	2		50 ml.
	8		100 ml.
	4		250 ml.
	3		400 ml.
	2		600 ml.
	1		1000 ml.
Bottles	6	Metric shelf bottles	1000 ml.
	2	resistance glass	500 ml.
Brush	1	Test tube, wire handle, tufted end, black bristle	
Burettes	1	Schellbach blue line Exax, straight stopcock	10 ml.
	2		50 ml.
Burette Clamp	1	Castaloy	
Burette Support	1	Porcelain base	
Burner	1	Tirrill type, medium stock	
Cuvettes	23	Round test tubes for matching	19 x 105 mm.
Cylinder	1	Graduated, glass blue line	100 ml.
Distilling Apparatus	1	All glass distilling flask and condenser	2000 ml.

Drying dishes	12	Aluminum dishes with covers	6.3 cm. diam. 1.8 cm. depth	
Filter paper	5 boxes	Whatman No. 12, folded	12.5 cm.	
	3 boxes	Whatman No. 12, folded	18.5 cm.	
Flasks	8	Erlenmeyer, pyrex	125 ml.	
	2		60 ml.	
		Volumetric, Exax, glass stoppered		
	4		50 ml.	
	8		100 ml.	
	2		250 ml.	
2	500 ml.			
2	1000 ml.			
Funnels	2	Pyrex glass, ribbed	10.0 cm. diam.	
	8		7.5 cm. diam.	
Funnel support	2	Wooden support with metal clamp to hold four funnels each		
pH Meter	1	Beckman laboratory model G.		
Pipettes		Pipettes, Exax, volumetric transfer		
	1		1 ml.	
	1		2 ml.	
	1		3 ml.	
	6		4 ml.	
	4		4 ml.	
	1		5 ml.	
	1		10 ml.	
	5		25 ml.	
	1		50 ml.	
	1		100 ml.	
			Graduated transfer pipettes	
	1			1 ml.
	1			5 ml.
1	10 ml.			
Powerstat	1	Type 116, 0 - 135 volts Variable resistor Superior Electric Co.		

Item		Description	Size
Recorders	1	Portable, Bristol Humidigraph 4 inch chart Model 4044	
	1	Portable Bristol Temperature recorder 4 inch chart Model 144	
Spectrophotometer	1	Coleman Model 11, Universal	
Stirrer	1	Laboratory stirrer, 110 volts, 60 cycle	
Supports	1	Ring stand, rectangular base, cast iron	
	1	Test tube, wire rack, galvanized to hold 40 test tubes	
	1	Test tube, wooden rack, to hold 10 test tubes	
	1	Cuvette rack, rubber coated to hold 12 cuvettes	
Test tubes	15	Pyrex glass with rims	16 x 150 mm.
Waring blender	1	Motor 100 volts	
Blendor jars	4	Glass with plastic tops	1 quart capacity
Water bath	1	Constant temperature bath Fisher unitized	