THE EFFECT OF WINTER STORAGE ON THE ASCORBIC ACID CONTENT OF APPLES, CABBAGE, ONIONS AND POTATOES GROWN IN MICHIGAN

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THE EFFECT OF WINTER STORAGE ON THE ASCORBIC ACID CONTENT OF APPLES, CABBAGE, ONIONS AND POTATOES GROWN IN MICHIGAN

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THE EFFECT OF WINTER STORAGE ON THE ASCORBIC ACID CONTENT OF APPLES, CABBAGE, ONIONS AND POTATOES GROWN IN MICHIGAN

PURPOSE OF THE STUDY

Many people living in Michigan depend upon their vegetable gardens and orchards for a large part of their food supply for the year. Some of the foods they are able to produce which can be stored for a period of time into the winter in the raw state are apples, cabbage, onions and potatoes.

One of the important contributions which these foods make to the diet in order to maintain an adequate nutritional state is the vitamin, ascorbic acid. The purpose of this study is to give information as to the actual ascorbic acid content of these foods under different conditions of winter storage. This information is of value in the wiser recommendation of the use of some Michigan-grown foods in the daily diet. This study also adds to the general information concerning the ascorbic acid content of common foods.

REVIEW OF LITERATURE

Literature published concerning the effect of winter storage on the ascorbic acid content of apples indicates that differences are due to the variety of apple and the type of storage.

Kohman, Eddy and Carlsson, 1924, following the method of Sherman, LaMer and Campbell, fed raw cold-stored apples to guinea pigs. The animals on all the levels fed (5, 6 and 7.5 grams of apple daily) were losing weight on the twentieth day of the experiment. The stored apples were replaced by fresh, early summer apples purchased on the open market and considerable improvement in the animals was noted. They concluded that raw apples in storage gradually lose their antiscorbutic potency.

Bracewell, Hoyle and Zilva, 1930, found a variation in the antiscorbutic activity of apples of different varieties after feeding them to guinea pigs. They reported that the variety of English apple known as Bramley's Seedling was markedly more active antiscorbutically than other varieties studied and there was little loss in the vitamin C content of apples stored at 1^{o} C. in the air for about three months. Bracewell, Kidd, West and Zilva, 1931, continued the study of the English apple, Bramley's Seedling, stored at 3^{o} C. in the air for five months and found that it did not lose any of its antiscorbutic activity. They also stored some of Bramley's Seedling apples at 1° C. They stated that in this variety, a displacement of the metabolic balance of the tissue occurred during storage at 1° C. eventually leading to low temperature tissue breakdown, whereas at 3° C. the metabolic activities of the fruit proceeded normally.

Baldwin and McIntosh apples were studied by Fellers, Isham, and Smith, 1932, by feeding radial sections to guinea pigs. Apples held in storage from October to February lost about twenty per cent of the vitamin C content when compared with the amount present in the freshly picked fruit. Fellers, Cleveland and Clague, 1933, reported that Baldwin apples lost about twenty per cent of the vitamin C content in four to six months' storage and forty per cent in eight to ten months of storage.

The vitamin C content of twenty-one varieties of apples grown in Massachusetts was determined by Smith and Fellers, 1934. The apples were stored at 33°-34° F. until fed to guinea pigs. They found that the Northern Spy apple was particularly high in vitamin C content. They reported that after two months' storage, five grams of the Northern Spy apple was a probable protective dose in preventing any evidences of scurvy in the guinea pigs.

This compares favorably with the minimum protective dose of the richest variety of apple yet reported--three grams of the Bramley's Seedling apple, according to Bracewell, et. al., 1930, 1931.

Batchelder, 1934, determined the vitamin C in Delicious apples before and after storage by feeding radial sections to guinea pigs. The vitamin C was determined in the fall, (October to December); in the winter, (January to March); and in the spring, (March to May). The apples were stored in two ways; referred to as cold storage and common storage. Cold storage apples were stored at 32° F., while common storage apples were stored at 45° F. The results of this study showed that cold storage preserved the vitamin C content of Delicious apples while common storage permitted some destruction. No loss of vitamin C was evident after six months of storage at 32° F. A loss of one-sixth or less occurred during the first three months of storage at 45° F., and a loss of about one-fourth during storage for six months at this temperature. The minimum protective dose was found to be twenty to twenty-five grams for the Washington Delicious apple.

Batchelder and Overholser, 1936, report that Winesap and Delicious apples fed in radial sections to guinea pigs showed greater loss of vitamin C when stored at 40° F.

for six months than the apples stored at 32° F.

Manville, McMinis and Chuinard, 1936, reported that the Northern Spy apple contained 6.0-7.5 units of vitamin C per ounce. They stated that there was a tendency for vitamin C to decrease with storage but that this tendency in apples was not as pronounced as in some other fruits, such as pears. They reported that the amount of vitamin C destruction varied with the kind of apple---being less stable in the Jonathan than in the Delicious apple. Arkansas Black and Delicious apples showed no variation due to storage in this study.

There was general agreement in the literature that the ascorbic acid content of cabbage decreased during a period of winter storage.

Eddy and Kohman, 1924, in the study of the vitamin C content of canned cabbage, fed raw cabbage to their control guinea pigs according to the method of Sherman, LaMer and Campbell. They stated that for the purpose of accurate comparison, they attempted to use throughout the experimental period of eighty days, cabbage taken from the same lot that was canned. They stored the cabbage in a cellar room. The feeding experiments were begun in December and approximately a month later, in January, they found that the usual amount of one gram per day of this lot began to lost potency. The outer leaves of the cabbage were wilted but the inner leaves which they fed, seemed crisp. They then substituted fresh cabbage obtained in the market and reported that they noted again the usual potency of one gram of cabbage per day. The authors of this article interpreted this to mean that there was some destruction of vitamin C during storage.

The effect of storage on the ascorbic acid content of cabbage was determined by Gould, Tressler and King, 1936, using the chemical titration method of Bessey and King. The cabbage was harvested at optimum maturity and some was stored at room temperature, 21-23° C.; some was stored at 8-9° C.; and some at 1-3° C. They reported that the cabbage stored at room temperature was spoiled in two months but the cabbage stored at the lower temperatures was studied for three months. Every two weeks a head of cabbage was removed from each type of storage and analyzed. Their results indicated that there was a slow loss of vitamin C during storage and that the higher the temperature of storage, the more rapid the loss.

Mayfield and Richardson, 1940, studied the effect of winter storage on the vitamin content of Danish Ballhead cabbage. This cabbage, the main variety grown in Montana for winter use, was stored as it would be in many farm homes--in a vegetable room in a home basement. They reported that the moisture content changed only slightly during storage. The outer leaves of the cabbage became withered and parchment-like but the inner leaves remained crisp and firm. They determined the ascorbic acid content by the biological method and the chemical titration method and found that these methods agreed rather closely. They concluded that Danish Ballhead cabbage was an excellent source of ascorbic acid and that winter storage caused a small loss (twenty-five per cent) in its vitamin C content.

The evidence reported in the literature indicated that the effect of winter storage on the ascorbic acid content of raw onions was variable.

Biological and chemical methods of testing were used by Mayfield and Richardson, 1940, to determine the effect of winter storage on the ascorbic acid content of the variety of onions, Sweet Spanish. The onions were stored in a vegetable room such as might be found in many farm homes. They found no change in the ascorbic acid content of this variety of onions from fall to spring.

Murphy, 1941, determined the ascorbic acid content of sixteen varieties of onions grown in Maine. The data showed that losses ranged from twenty-three to sixty-four per cent the first month of storage (October - November). The second month, (November-December), the losses ranged from zero to fifty-four per cent of the amounts observed after one month of storage. During the third month (December - January), only slight losses were noted for most varieties.

The ascorbic acid content of raw potatoes varies during winter storage due to varietal differences and to conditions of storage.

Woods, 1935, reported that the mature Russet Burbank (or Netted Gem) potato grown in Idaho does not change in vitamin C content to any marked degree when stored at 40-50° F. for three to eight months. The storage conditions approximated the conditions of storage of potatoes on the farms of Idaho. The potatoes kept in an edible condition until the middle of June.

Thiessen, 1936, studied the effect of storage at 38-42° F. upon the vitamin C content of the Bliss Triumph potato grown in Wyoming. The results of feeding guinea pigs the potato indicate that the vitamin C content of this particular potato was depleted about one-half during the storage period of six to nine months.

The changes in the ascorbic acid content of potatoes stored at three different temperatures were reported by Pett, 1936. Potatoes, newly dug, were stored at 15°, 10°, and 5° C. The ascorbic acid content was determined by chemical titration. There was rapid loss in the vitamin C content of the potatoes in this study at all three temperatures of storage, although most at the highest temperature. The rate of loss was reported to be dependent upon the initial value; and the ascorbic acid tended to come to a certain level after which a much slower decrease occurred.

Mayfield, Richardson, Davis and Andes, 1937, reported the effect of two types of winter storage on the vitamin content of two varieties of Potatoes grown in Montana--the Russet Burbank and the Bliss Triumph. Two contrasting types of storage cellars were used: one type had controlled temperatures from 37-46^{o} F., with controlled humidity, light and ventilation; the other type was a vegetable room in a private house with conditions typical of home storage with no attempt made to regulate temperature or humidity. The ventilation and light in this room was supplied by a north basement window. The results of this study showed that the moisture content for each variety showed very little change as storage time progressed. Six months' storage under controlled conditions caused no loss of vitamin C in the raw Netted Gem potato according to biological tests, but a one-third loss according to the results obtained with the chemical titration method of Bessey and King. Six months' storage in the warmer cellar caused practically no loss in the vitamin C content of the raw Netted Gem potato according to tests by both biological and chemical methods. These results agree with those reported by Woods, 1935. Six months' storage in the cool cellar caused a loss of one-third to one-half in the vitamin C content of the raw Bliss Triumph potato according to animal and chemical tests. This was in agreement with the results of Thiessen, 1936. Six months' storage in a warm dry cellar caused about twenty per cent loss of vitamin C in the raw Bliss Triumph potato, according to animal and chemical tests.

Variation in ascorbic acid content from tuber to tuber was found by Rolf, 1940, to be much smaller in potatoes that had been stored than in immature or new potatoes. The varieties, Green Mountain and Irish Cobbler were reported by Rolf to lose ascorbic acid during storage at 15.5° C. The losses were most rapid during the first few weeks of storage and thereafter the losses became more gradual.

Smith and Gillies, 1940, studied the vitamin C content of eighteen varieties of potatoes by the chemical titration method. These results showed that when the potatoes were stored at a temperature between 2-8° C., in every case there was a substantial decrease in the amount of ascorbic acid during the period of storage.

Esselen, Lyons and Fellers, 1942, reported the effect of storage on the ascorbic acid content of eight varieties of potatoes grown in Massachusetts. They checked the chemical titration method of Bessey and King against the biological method of Sherman, LaMer and Campbell and found close agreement in the first cases so the remainder of the tests were done by the chemical method. The potatoes were stored in two different ways for five months. One set of each variety was kept at 2.2° C. A second set of each variety was kept in dry underground storage where the temperature was largely controlled by climatic conditions and the temperature ranged from 4.4° C. - 10° C. The second type of storage would be similar to cellar or common storage on a farm. The results that were obtained varied. They found that in two varieties, the Chippewa and the Katahdin, the cold storage potatoes had higher ascorbic acid content than did the potatoes stored at the warmer temperature. In four varieties the reverse was true and in two varieties there was no difference. They also reported that storage seems to cause leveling off of the differences between varieties in ascorbic acid content.

EXPERIMENTAL PROCEDURE

The foods chosen for this study were of four different types:

a. a pome--Northern Spy apple

b. a leafy vegetable--Danish Ballhead cabbage

c. a bulb--Yellow Globe onion

d. a tuber--Chippewa potato.

These foods were chosen because of their storage qualities and because they are common varieties grown in Michigan which are suitable for winter storage, according to Seaton, 1941, 1942.

The foods were purchased in November and early December of 1942 and each was divided into two sets. One set of each food was placed in a large refrigerator in which the temperature ranged from 3-5° C. for the entire storage period. The foods stored in this way will be hereafter referred to as refrigerator-stored.

The other sets of cabbage, apples and potatoes were stored in a cement-lined basement room with one small west window which gave ample ventilation. The other set of onions was stored in an attic room. Directions for this type of storage were obtained from Seaton, 1941, 1942. The temperature in these two rooms was dependent largely upon climatic conditions. This type of storage simulated the storage conditions that could likely be attained in almost any home with very little effort. The foods stored in this manner will hereafter be referred to as home-stored.

Reduced ascorbic acid in these foods was determined according to the method of Bessey, 1938, with modifications according to Morell, 1941.

> Each of the foods was sampled by cutting radial sections from opposite sides with a glass knife. Samples (twenty-five grams weighed to the nearest 0.1 gram) were cut and immediately placed under approximately 100 ml. of 3% metaphosphoric acid. The immersed samples were ground in the Waring Blendor for exactly two minutes. The extract was then transferred quantitatively to a 250 ml. volumetric flask and was made up to volume with 3% metaphosphoric acid. This extract was filtered through a folded filter paper (Whatman #12) into small erlenmeyer flasks. Aliquots of this filtrate were transferred to suitable volumetrics so that 50 ml. of diluted extract contained 100-450 micrograms of ascorbic acid. These aliquots were then made up to volume with 3% metaphosphoric acid and enough sodium citrate buffer to bring the pH of the extract to 3.5-3.6. A blank solution was made by adding enough sodium citrate buffer to 3% metaphosphoric acid to bring the pH to 3.5-3.6.
>
> The Coleman Universal Spectrophotometer, Model 11, was used to determine the amount of ascorbic acid in the buffered extract. The wave length of the spectrophotometer was set at 518-520 and the galvonometer was set at 100 when a tube of 4 ml. of the blank solution plus 4 ml. of dye (2-6 dichlorophenol-indophenol), decolorized by adding a crystal of ascorbic acid, was placed in the beam of light. Four ml. portions of the dye were placed in dry, matched test tubes. Into this dye was blown from a pipette, 4 ml. of the buffered plant extract. The tube was placed in the beam of light within five seconds. Readings on the galvonometer were taken at exactly 15 seconds and again at 30 seconds after the introduction of the extract into the dye. A crystal of ascorbic acid was

added to the test tube to completely reduce the dye, and a third reading was taken. These readings were referred to as Gs1, Gs2 and Gsr, respectively. To correct for the color and the turbidity of the extracts measured, the machine was then set to give a galvonometer reading that was the same as the Gsr reading. A tube of 4 ml. of the dye plus 4 ml. of blank solution was read exactly 15 seconds after mixing. This reading was referred to as Gb. Three readings were taken for each extract, and an average of the three readings was used to determine the ascorbic acid in the sample.

The following formula was used in calculating the amount of ascorbic acid present in the extract:

Mg. ascorbic acid per ml. of extract = K x Log Gs-Log Gb

$$Gs = Gs1 - (Gs2 - Gs1)$$

K = 0.0311 (determined by iodine titration according to Bessey and King, 1933)

The determinations of reduced ascorbic acid were made before the foods were placed in storage and once a month thereafter until the close of the study (or until the foods were not edible). Once a month six apples, six onions and six potatoes were taken from each storage place and duplicate determinations were carried out on each of the foods. Each month three heads of cabbage were removed from each type of storage and four determinations were done on each head. Apples and potatoes were analyzed without peeling. The dry outer layers of the onion and the outer, inedible portions of the cabbage heads were removed before sampling.

Moisture content of the foods analyzed for ascorbic acid was determined each month. Five to ten gram samples were dried for twenty-four hours at a temperature ranging from 90-100° C. This procedure made it possible also to calculate percentage solids or dry weight of the samples.

The last month of the study (April, 1943) ascorbic acid determinations were carried out on the refrigerator-stored foods only. Total ascorbic acid--reduced ascorbic acid plus dehydroascorbic acid--was determined on the extracts of these foods according to the method of Bessey, 1938. Hydrogen sulfide was slowly bubbled through the buffered extract for fifteen minutes and the excess hydrogen sulfide was removed by bubbling nitrogen through the extract after two hours of standing at room temperature.

The data were analyzed statistically by determining "t" values according to the method of Fisher, 1936. The figures used in the statistical analyses were those calculated on the basis of milligrams of ascorbic acid per 100 grams of dry weight.

RESULTS

The apples, potatoes and onions that were refrigerator-stored were in good condition for the entire period of the study. The cabbage that was refrigerator-stored was not in good condition at the close of the study in April. When the cabbage was used in March, the outer leaves were dry and dark in color and in April the condition was even more advanced as more leaves were involved and some mold was evident. However, when that portion of the cabbage was removed, the inner leaves which were used as samples were firm and light in color.

The home-stored apples were not in an edible condition in March. The apples were not in good condition in the month of February but at that time enough apples were whole and unspoiled for use in the analyses. The home-stored cabbage was also not edible in March. The cabbage was sprouted and the outer leaves were either dry or moldy and spoilage was so great that no determinations could be done on the home-stored cabbage in March. The home-stored onions were showing evidences of sprouting in February and the sprouts were even more evident in March. They were, however, still satisfactory for use. The home-stored potatoes were slightly shriveled in March and April but they still seemed satisfactory for use.

There were no sprouts evident on the potatoes at the end of the storage period in April.

Apples: The mean values of the reduced ascorbic acid content of Northern Spy apples on the basis of moist and dry weight are given in Table I. The mean values for each month are based on twelve determinations done on six apples.

TABLE I

The Effect of Storage on the Reduced Ascorbic Acid Content of Northern Spy Apples

Month	Refrigerator-Stored		Home-Stored	
	Mg./100 g. moist wt.	Mg./100 g. dry wt.	Mg./100 g. moist wt.	Mg./100 g. dry wt.
December*	10.65	64.14	10.65	64.14
January	11.21	70.86	11.80	73.21
February	11.40	75.29	12.54	77.32
March	12.33	78.03	-----	-----
April	10.95	69.68	-----	-----

*The values for December are the quantities of reduced ascorbic acid that were present in the apples before being placed in storage.

A straight line was fitted, by the method of least squares, to the data concerning the reduced ascorbic acid content of the apples stored in the refrigerator from December through March. The slope of that line was significantly different from zero. There was no significant difference between the average ascorbic acid values of

December and January; of December and February; or of December and March. The difference between the mean values of December and March were almost significant, however, and if the trend had continued as indicated by the slope of the line, the changes in ascorbic acid probably would have been significant.

A straight line also was fitted to the data on the apples which were home-stored from December through February. The slope of that line was likewise significant. The mean ascorbic acid values of January and February were found to be not significantly different from the mean value of December in the home-stored apples.

The data obtained from the home-stored apples were compared to the data on the refrigerator-stored apples. The slopes of the straight lines were not significantly different. Neither was there any significant difference between the ascorbic acid content of the refrigerator-stored apples and the home-stored apples at any time during the storage period. The actual amounts of reduced ascorbic acid present in the apples during the storage period are shown in Figure I. There is a slightly higher content noted in the home-stored apples but that difference is not significant.

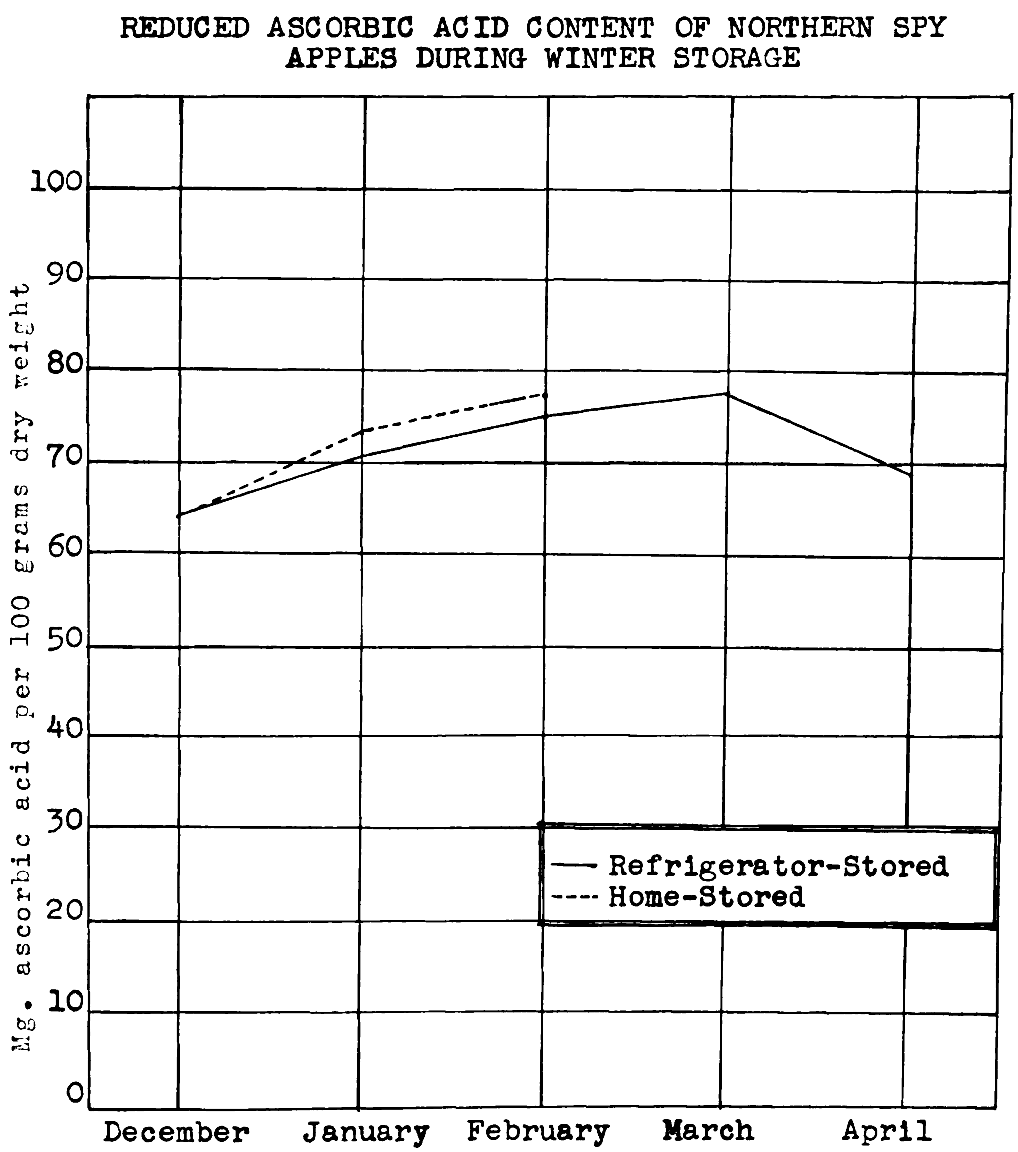
REDUCED ASCORBIC ACID CONTENT OF NORTHERN SPY
APPLES DURING WINTER STORAGE
Mg. ascorbic acid per 100 grams dry weight
100
90
80
70
60
50
40
30
20
10
0
December
January
February
March
April
Refrigerator-Stored
Home-Stored

Figure I

<u>Cabbage</u>: The average values of the reduced ascorbic acid content of Danish Ballhead cabbage on the basis of moist and dry weight are given in Table II. The average quantities for each month for each method of storage were based on twelve determinations done on four heads of cabbage.

TABLE II

The Effect of Storage on the Reduced Ascorbic Acid Content of Danish Ballhead Cabbage

Month	Refrigerator-Stored		Home-Stored	
	Mg./100 g. moist wt.	Mg./100 g. dry wt.	Mg./100 g. moist wt.	Mg./100 g. dry wt.
December*	48.74	602.4	48.74	602.4
January	46.36	558.5	42.87	486.2
February	44.28	507.0	45.71	383.8
March	29.03	376.5	-----	-----
April	27.18	372.1	-----	-----

*The values for December are the quantities of reduced ascorbic acid that were present in the cabbage before being placed in storage.

By the method of least squares, a straight line was fitted to the data concerning the ascorbic acid content of the cabbage stored in the refrigerator. The slope of that line was not significantly different from zero. There was no significant difference between the mean of December and the means of January and February. There was a significant difference, however, between the mean of December and the means of March and April.

The data concerning the reduced ascorbic acid content of home-stored cabbage showed an almost straight-line relationship between the means of the three months' determinations. The ascorbic acid content of the cabbage in January was not significantly different from the content in December. However, the amount present in February was significantly less than that which was present in December.

When the data on the reduced ascorbic acid content of the refrigerator-stored and the home-stored cabbage were compared, it was found that there was no significant difference in the values of the cabbage due to method of storage in January, but in February there was a significant difference. The home-stored cabbage in February contained a smaller amount of reduced ascorbic acid than did the cabbage that was refrigerator-stored.

The comparison of the mean quantities of reduced ascorbic acid in the cabbage during the storage period may be noted on the graph shown in Figure II.

Onions: The average values of the reduced ascorbic acid content of Yellow Globe onions during the storage period on the basis of moist and dry weights are given in Table III. The means for each month were based on twelve determinations done on six onions from each type

REDUCED ASCORBIC ACID CONTENT OF DANISH
BALLHEAD CABBAGE DURING WINTER STORAGE

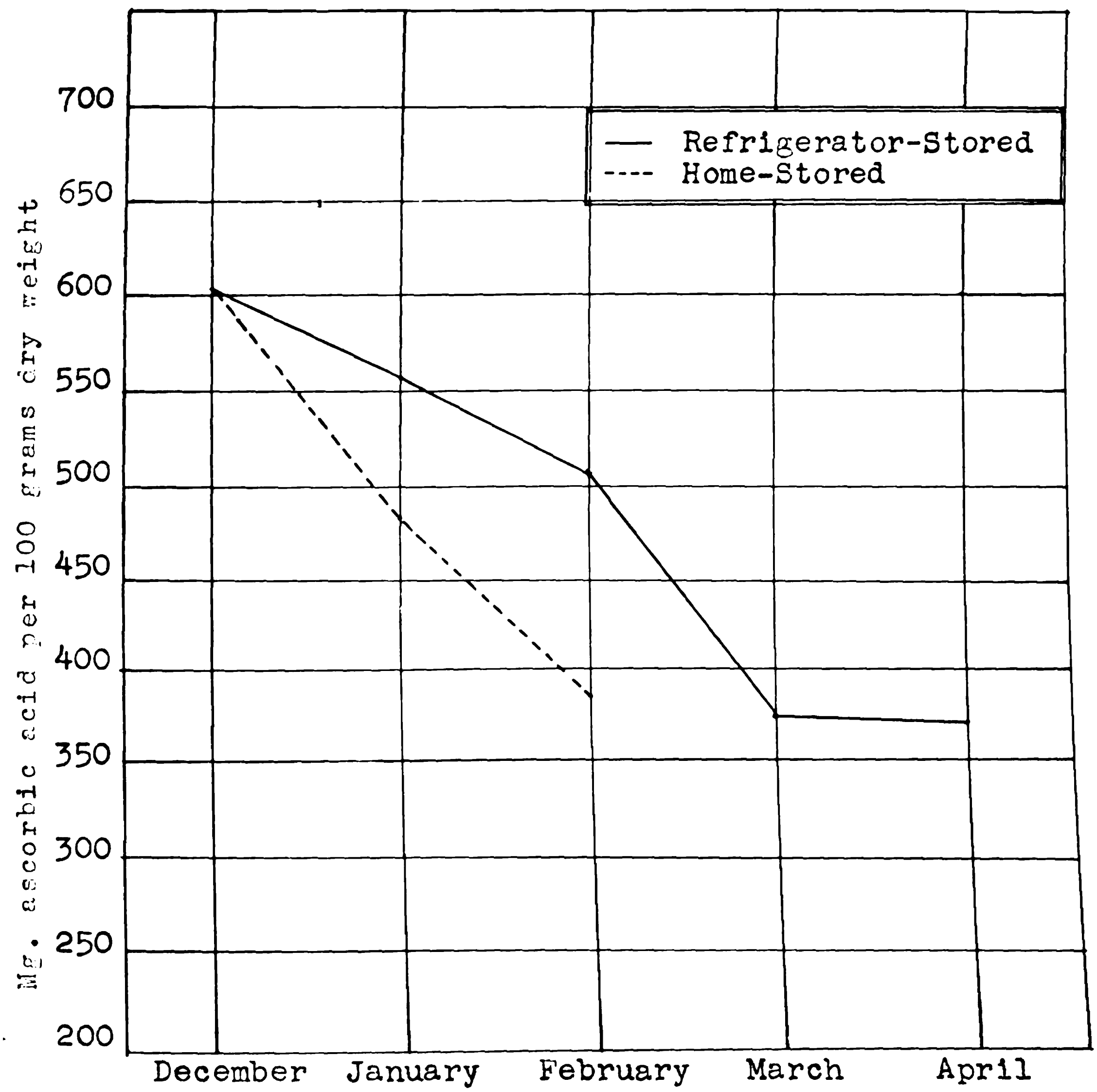

FIGURE II

of storage.

TABLE III

The Effect of Storage on the Reduced Ascorbic Acid Content of Yellow Globe Onions

Month	Refrigerator-Stored		Home-Stored	
	Mg./100 g. moist wt.	Mg./100 g. dry wt.	Mg./100 g. moist wt.	Mg./100 g. dry wt.
November*	6.407	78.81	6.407	78.81
December	5.599	69.26	6.076	67.81
January	5.367	65.21	5.885	79.57
February	5.878	69.95	6.235	85.42
March	6.139	73.11	6.441	85.47
April	6.051	77.81	-----	-----

*The values for November are the quantities of reduced ascorbic acid that were present in the onions before being placed in storage.

The onions that were refrigerator-stored showed a significant difference in reduced ascorbic acid content in December from those sampled in November at the beginning of the storage period. That difference was a decrease in the ascorbic acid content. In January, there was again a significant difference when compared with the amount present in November and there was a further decrease in the ascorbic acid content. In February, the amount of reduced ascorbic acid seemed to increase when compared to that present in January, but the difference was not significant when compared to either the January value or to the amount present at the beginning of the storage period in November. The means of the March and April samples were also not significantly different from the mean of the November samples. The mean reduced ascorbic acid value of the April samples was approximately the same as the mean of the November samples. The graphic comparison of the mean reduced ascorbic acid contents of the refrigerator-stored and the home-stored onions may be noted in Figure III.

The home-stored onions showed a change in the reduced ascorbic acid content that was significantly less in December than in November. The ascorbic acid content, however, increased again and by January, the mean value of reduced ascorbic acid was significantly greater than that present in December. However, this mean of the January samples was not significantly different from the mean of the samples analyzed at the beginning of the storage period--in November. The home-stored onions did not show a significant difference in the ascorbic acid content from the November mean in the last month's determinations--in March.

When comparing the results of the home-stored and refrigerator-stored onions with each other, there was only one month when the one type of storage was significantly different from the other type of storage as far as

REDUCED ASCORBIC ACID CONTENT OF YELLOW GLOBE ONIONS DURING WINTER STORAGE

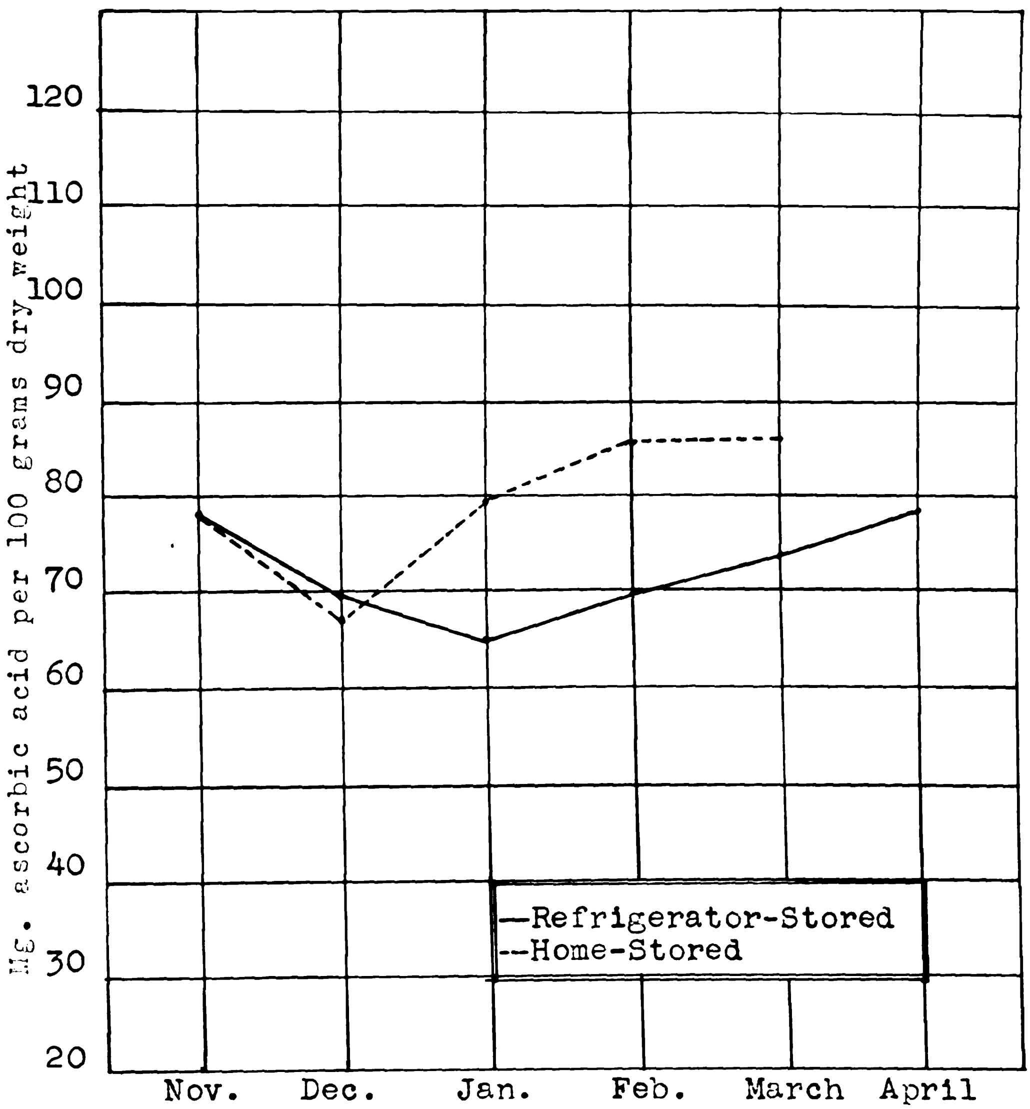

Figure III

the content of reduced ascorbic acid was concerned. That month was January and at that time, the ascorbic acid content of the home-stored onions was significantly higher than that of the refrigerator-stored onions.

Potatoes: Table IV shows the average values of reduced ascorbic acid in Chippewa potatoes during a period of winter storage. The values given for each month are based on the six potatoes from each type of storage on which the twelve determinations were done.

TABLE IV

The Effect of Storage on the Reduced Ascorbic Acid Content of Chippewa Potatoes

Month	Refrigerator-Stored		Home-Stored	
	Mg./100 g. moist wt.	Mg./100 g. dry wt.	Mg./100 g. moist wt.	Mg./100 g. dry wt.
December*	11.07	59.49	11.07	59.49
January	7.373	40.67	9.377	44.06
February	6.670	35.77	6.662	33.03
March	5.416	27.27	4.462	20.07
April	4.089	20.57	-----	-----

*The values for December are the quantities of reduced ascorbic acid that were present in the potatoes before being placed in storage.

A straight line was fitted to the entire data obtained from determining the reduced ascorbic acid in the refrigerator-stored potatoes. The slope of this line was found to be significantly different from zero.

There was a significant difference between the mean of December and the mean of January. Since there was a significant difference between the means of December and January, there was also a significant difference between the means of December and every other month as the ascorbic acid content was decreasing with each month of storage in an almost straight line relationship.

A straight line was also fitted to the data concerning the reduced ascorbic acid content of the home-stored potatoes. The slope of this line was highly significantly different from zero. The difference between the means of December and January was found to be not quite significant but the difference between the mean of December and the means of February and March was found to be definitely significant.

The graph shown in Figure IV denotes the decreasing value of reduced ascorbic acid during the period of winter storage of the potatoes.

There was no significant difference between the reduced ascorbic acid content of the home-stored potatoes and the refrigerator-stored potatoes in January and February but in March, the refrigerator-stored potatoes had a significantly higher ascorbic acid content.

The slopes of the straight lines, fitted to the data concerning the refrigerator-stored and the home-

REDUCED ASCORBIC ACID CONTENT OF CHIPPEWA POTATOES DURING WINTER STORAGE

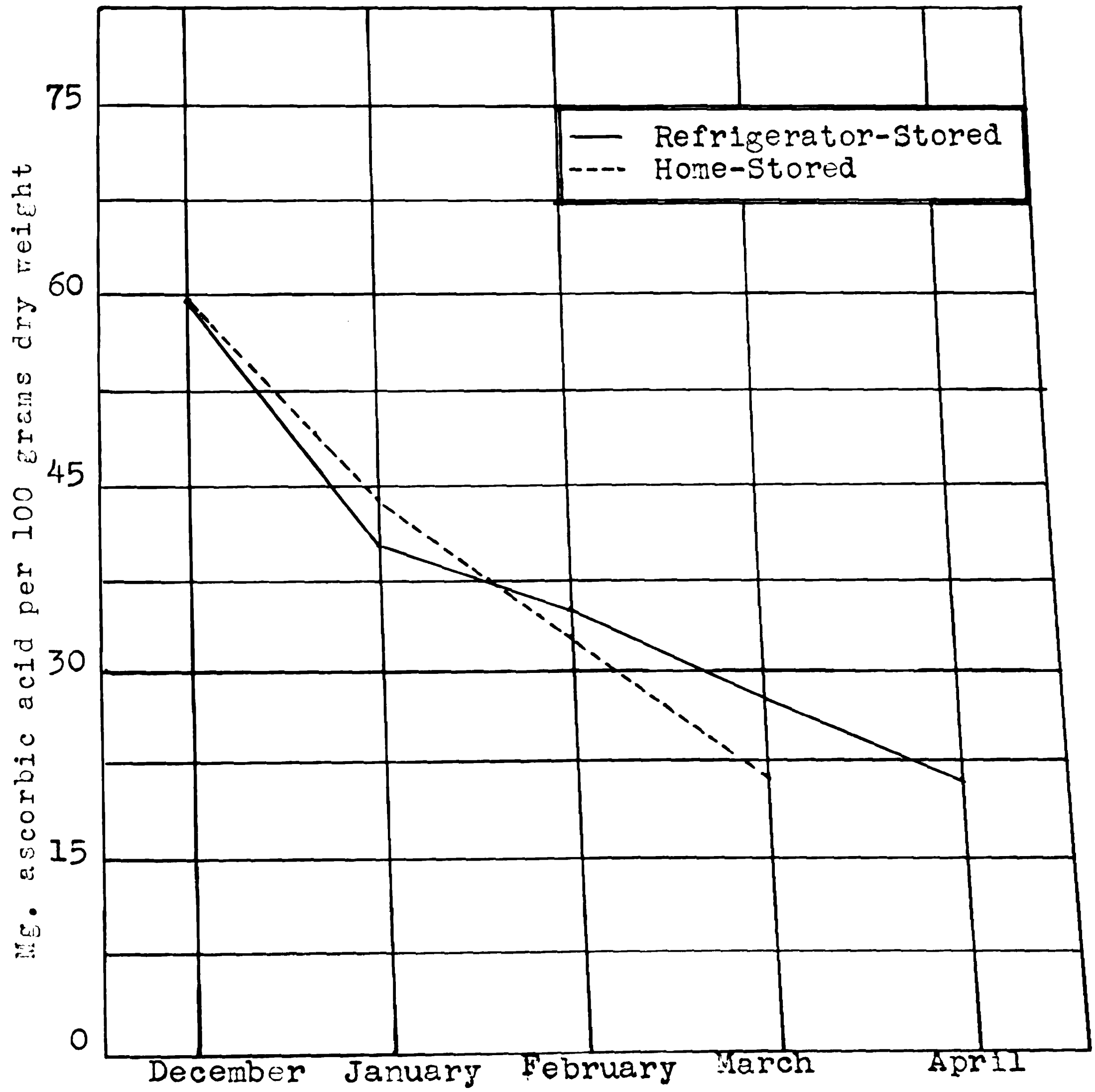

Figure IV

stored potatoes by the method of least squares, were significantly different.

Total versus Reduced Ascorbic Acid: The foods that were sampled in April were tested for total ascorbic acid as well as for reduced ascorbic acid. A comparison of the values for each food are given in Table V. The difference in the total and reduced ascorbic acid content was significant in the potatoes and the cabbage, but it was not significant in the onions and the apples. There was some increase noted in all the samples, however.

TABLE V

Ascorbic Acid Content of Refrigerator-Stored Foods Before and After Treatment with Hydrogen Sulfide

Food	Reduced		Total	
	Mg./100 g. moist wt.	Mg./100 g. dry wt.	Mg./100 g. moist wt.	Mg./100 g. dry wt.
Apples	10.95	69.68	12.98	83.08
Cabbage	27.18	372.1	32.36	443.0
Onions	6.051	77.81	7.045	90.61
Potatoes	4.089	20.57	6.322	31.80

Moisture: The moisture content of the four foods studied showed only very slight variation during the period of storage. The moisture content for the first month and for the last month of storage are shown in Table VI.

TABLE VI

Moisture Content of Apples, Cabbage, Onions and Potatoes

Food	Percentage Moisture			
	Refrigerator-Stored		Home-Stored	
	1st month	Last month	1st month	Last month
Apples	83.40	84.29	83.40	83.68
Cabbage	91.91	92.70	91.91	88.09
Onions	91.87	92.22	91.87	92.47
Potatoes	81.40	80.12	81.40	77.77

DISCUSSION OF RESULTS

Apples: The ascorbic acid content of the Northern Spy apples in this study compared favorably with the values reported by Booher, Hartzler and Hewston, 1942, and these apples did not decrease in their ascorbic acid content (reduced) during a period of winter storage of four months in a refrigerator at a temperature near 4° C. Home-stored apples of the Northern Spy variety also showed no decrease in reduced ascorbic acid content during a period of two months' storage when storage temperature depended largely upon climatic conditions.

Previous work with apples that has been reported in the literature indicated that some varieties lost ascorbic acid when stored and others did not. Bramley's Seedling, an English variety of apples, when stored at 3° C. for five months, lost none of its antiscorbutic activity, according to Bracewell, et al., 1930, 1931. Delicious apples that were stored at 0° C. lost none of their vitamin C after six months' storage, was reported by Batchelder, 1934. Fellers, et al., 1932, 1933, reported that the Baldwin and the McIntosh apples lost about twenty per cent of the vitamin C content in four to six months' storage. Batchelder and Overholser, 1936, found that storage at 40° F. resulted in greater loss of vitamin C in Winesap and Delicious apples than did storage at 32° F.

The Northern Spy apple is considered to be a very good source of ascorbic acid when compared with other varieties of apples, according to Smith and Fellers, 1934. There was, however, no information available in the literature at the present time as to the trend in the ascorbic acid content of the Northern Spy apples during a period of winter storage. Manville, McMinis, and Chuinard, 1936, reported that there is, no doubt, a tendency for the ascorbic acid content of apples to decrease with storage but they gave no evidence of actual changes in the ascorbic acid content of the Northern Spy apple during a period of storage.

On the basis of the evidence reported in this study, it seems possible that the Northern Spy apple grown in Michigan does not lose ascorbic acid during a period of winter storage. It may be that it compares with the Bramley's Seedling apple in that respect.

The Northern Spy apples in this study showed no differences in ascorbic acid content that were significant due to the two different types of storage. Whether any differences might have shown up later if the home-stored apples had kept longer is a matter of question.

Cabbage: Evidence reported in this study showed that

Danish Ballhead cabbage grown in Michigan does lose ascorbic acid when stored and the higher the temperature of storage, the more rapid the loss.

The refrigerator-stored cabbage lost ascorbic acid at a slower rate than did the home-stored cabbage which was stored at the higher and more widely varying temperature. These results agree with the literature that has been reported to date on the effect of storage on the ascorbic acid content of cabbage. Mayfield and Richardson, 1940, studied the effect of a type of home storage on the vitamin C content of Danish Ballhead cabbage that was grown in Montana. They reported that the loss in vitamin C was twenty-five per cent. This is somewhat less than the loss of the Michigan-grown home-stored cabbage in which the loss was approximately thirty-six per cent, on the basis of dry weight.

The refrigerator-stored cabbage that was stored the same length of time as was the home-stored cabbage lost approximately sixteen per cent. The refrigerator-stored cabbage lost as much ascorbic acid after four months of storage as the home-stored cabbage did in two months--approximately thirty-eight and thirty-six per cent, respectively.

The results of this study also agree with the results reported by Gould, Tressler and King, 1936, who found that a slow loss of vitamin C occurred during the storage of cabbage and also that the higher the temperature of storage, the more rapid the loss of the vitamin.

Yellow Globe onions: The onions analyzed in this study showed that there was a decrease in the ascorbic acid content of the onions during the first month of storage for the home-stored onions and during the first two months of storage for the refrigerator-stored onions. After this period of decrease in ascorbic acid content, there was a definite increase in that ascorbic acid content. This increase occurred first in the home-stored onions, followed a month later by an increase in the ascorbic acid content of the refrigerator-stored onions at a somewhat slower rate. At the end of the period of study, there was no significant difference between the ascorbic acid content when compared with the first month's content in the onions stored by either method.

The fact that no loss occurred when the ascorbic acid content present in the spring was compared with the amount of ascorbic acid present in the fall at the beginning of the study, compared favorably with the result of a study reported by Mayfield and Richardson, 1940, They analyzed the ascorbic acid content of the Sweet Spanish onion and found no loss during a period of storage from fall to spring. They had not followed the trend of the ascorbic acid content between the fall and spring periods of analyses, however.

The results concerning the Yellow Globe onion reported in this paper are comparable to the results concerning the Sweet Spanish onion in that no loss did occur from fall to spring by either method of storage.

Fluctuation in the ascorbic acid content of the Yellow Globe onions was noted in this study. Murphy, 1941, reported that in sixteen varieties of onions there was a loss from twenty-three to sixty-four per cent during the first month of storage; in the second month the losses over those reported for the first month were from zero to fifty-four per cent and the third month of storage in December and January, slight losses for most varieties were noted.

The Yellow Globe onions that were home-stored showed evidences of actual sprouting in February. The refrigerator-stored onions did not show any visible evidence of sprouting during the entire period of study. The significant difference in the ascorbic acid content that was observed in January in which the home-stored onions actually showed the higher ascorbic acid content might be answered by the fact that the onions stored at the warmer temperature of the attic storage had shown a change in their metabolism which was connected with their sprouting, even though the onions did not show any visible sprouts until the following month. It is a well-known fact that synthesis of vitamin C occurs during germination, according to Harris and Ray, 1933, and King, 1936. The sprouting of the onions might be likened to the germination of a seed.

The onions that were refrigerator-stored did not show this upward trend in ascorbic acid content until a month later. This fact might be due to the effect of the colder temperature of storage on the metabolism of the bulb.

Potatoes: The Chippewa potatoes showed definite decreases in the ascorbic acid content in both types of storage. The decrease was not markedly different in the two methods of storage until the month of March. The ascorbic acid content of the home-stored potatoes was significantly less by March than that of the refrigerator-stored potatoes. These results agree with those of Esselen, Lyons and Fellers, 1942, who stored the Chippewa potato in two ways--cold storage and common storage. They found that the Chippewa potato lost 63% in the common storage and 53% in the cold storage. When the losses of the Michigan-grown Chippewa potatoes were calculated on a percentage basis, the losses were found to be as follows: home-stored, 66% and refrigerator-stored, 56%. The period of storage of the Chippewa potatoes grown in Michigan was somewhat shorter, however.

Moisture: The moisture content of the Northern Spy apples changed very little during the period of storage. The moisture content of the home-stored cabbage in this study decreased during the period of storage. The change in the moisture content of the Danish Ballhead cabbage that was Michigan-grown was from 91.91% in the fall to 88.09% in the spring. This is comparable to the change reported by Mayfield and Richardson, 1940, who found that Danish Ballhead cabbage changed from 90% moisture in the fall to 87% in the spring. The moisture content of the Yellow Globe onions changed only slightly during the period of storage....91.87% in the fall to 92.47% in the spring. A larger increase than this was reported by Mayfield and Richardson, 1940, who stated that the moisture content of the Sweet Spanish onion changed from 89% in the fall to 91% in the spring. These figures refer to home-stored onions in both studies. The Chippewa potatoes showed a decrease in the moisture content during the period of storage. The change was greater in the home-stored potatoes than in the refrigerator-stored potatoes. The home-stored potatoes changed from 81.40% in the fall to 77.07% in the spring. The moisture content of the potatoes that were refrigerator-stored changed only from 81.40% to 80.12%

from fall to spring.

Table V shows the effect of treatment with hydrogen sulfide on the extracts of the foods analyzed in April. In each case, there was an apparent increase in the total ascorbic acid present. The amount of that increase was significant in the potatoes and the cabbage but it was not significant in the apples or in the onions.

The literature presents conflicting reports as to the necessity of treating extracts of plant tissues with hydrogen sulfide in order to determine total ascorbic acid.

The dye used in the chemical method for determining ascorbic acid measures only reduced ascorbic acid. This method does not measure any of the reversibly oxidized ascorbic acid which is biologically active. This reversibly oxidized ascorbic acid is also referred to as dehydroascorbic acid.

McHenry and Graham, 1935, state that in order to be certain that all the vitamin C present in the extract is determined by the chemical titration method, it is obvious that a preliminary treatment with hydrogen sulfide is essential.

Smith and Gillies, 1940, agreed with McHenry and Graham, since they almost always found a certain amount of dehydroascorbic acid present in potato tubers. They reported that there was no reason to suspect that other reducing substances are formed in tuber extracts by this treatment with hydrogen sulfide since they found little or no dehydroascorbic acid in tubers examined immediately after harvesting.

McHenry and Graham, 1935, reported that over fifty per cent of the total ascorbic acid in the onion tissue they analyzed was dehydroascorbic acid. They also found that approximately one-third of the total ascorbic acid in cabbage was dehydroascorbic acid.

Bessey, 1938, states that it is a significant fact that increased reducing power is usually found in those plant tissues which have been stored for some time, which are still in good condition, but which are not strictly fresh. He reported that potatoes, when first mature, show practically no dehydroascorbic acid, but after a few weeks' storage, the dehydroascorbic acid gradually represents a significant part of the slowly decreasing total ascorbic acid. Bessey found that in the Irish Cobbler potato, after ten months of storage, the dehydroascorbic acid represents one-third of the total.

It is true that not all research workers are convinced of the necessity of determining the dehydroascorbic acid content of tissue extracts. Harris and Olliver, 1942, reported that the stored potatoes they examined were the only food to contain significant amounts of dehydroascorbic acid. They reported that twelve per cent of the total ascorbic acid was dehydroascorbic acid, according to their determinations. They reported also that they believed that for all practical purposes, the treatment with hydrogen sulfide was hardly necessary.

Rolf, 1940, studying the varieties of potatoes--Green Mountain and Irish Cobbler, reported that at various times during the experiment, portions of extract were treated with hydrogen sulfide but no increased reducing activity was noted. This was confirmed by Esselen, et al., 1942, who found that no dehydroascorbic acid was present in the raw potatoes they studied.

If the significant increased values obtained in this study of Michigan-grown cabbage and Michigan-grown potatoes were actually dehydroascorbic acid, the loss of ascorbic acid would not be as great as has been indicated by the data obtained concerning the reduced ascorbic acid present in the cabbage and potatoes. There would still be a loss, however, as the total ascorbic acid figures for April are less than the ascorbic acid content figures found at the beginning of the storage period.

The author believes that the increase in reducing value of the extract after treatment with hydrogen sulfide in the case of each food studied may be due, in part, at least, to the presence of other reducing substances. It was reported by King, 1941, that many organic compounds gave reactions with hydrogen sulfide that were comparable to the reaction used for estimating dehydroascorbic acid. King stated that it was probable that much of the published data relative to the oxidized form of the vitamin may have been misinterpreted because of this interference by newly-formed reducing material that resulted from the hydrogen sulfide treatment. He reported also that many aldehydes, ketones and quinones gave rise to an interfering reaction when reduced with hydrogen sulfide and that the interference could not be eliminated by either titration or photo-electric colorimetric readings. He mentioned that there was a possibility for determining the total ascorbic acid by the use of the method of Roe, 1936, whereby the reduced and dehydroascorbic acid were measured by being converted into furfural. In this method the dehydroascorbic acid did not have to be reduced before measurement.

Ahmad, 1935, reported that it was undesirable to treat a complex natural material (such as extracts from foods) with hydrogen sulfide for such a long period of time as had been recommended (six hours) on account of the unknown changes that the treatment might induce.

Tests with some of the compounds which have been reported to react with hydrogen sulfide were conducted by Hochberg, Melnick and Oser, 1943. Their results showed that except for 2 methyl, 1-4 naphthoquinone, the interferences following the use of hydrogen sulfide were much greater in the visual titration method than in the photometric method. They found that pyruvic acid was one of the compounds which showed no interference. They believe that other interfering substances are normally found in biological materials in insufficient concentrations to interfere in the determination of total ascorbic acid. However, they did report that the reducing substances other than ascorbic acid were present in increased amounts in the extract after treatment with hydrogen sulfide.

Harris and Olliver, 1942, experimented with plant tissues and their results convinced them that, provided the correct procedure is adhered to for the determinations, dehydroascorbic acid determinations can be omitted. They believe that the large amounts of dehydroascorbic acid sometimes reported in fresh plant tissues are generally an artefact produced by oxidation. They reported that any fine chopping, mincing or shredding of the material with the supposed object of obtaining a homogeneous sample must be condemned because intra-cellular oxidases will thus be set free from many plant tissues with consequent rapid disappearance of the ascorbic acid. On the basis of their experiments, they stated that the amount of dehydroascorbic acid present in foods--even aging or stale foods--is generally so small as to be of little or no practical significance. This opinion is not in agreement with the opinion of Hochberg, Melnick and Oser, 1943.

The reason that the author of this article believes that there may be some other reducing substances present in the extract that was treated with hydrogen sulfide in April is this: The drift in the readings that were made at 15 seconds and 30 seconds after the buffered extract had been added to the dye when using the spectrophotometer is an indication of the presence of other reducing substances, according to Bessey, 1938. These reducing substances react more slowly than the ascorbic acid and their presence is indicated by the difference in the two readings. The drift in the readings of the extracts after having been treated with hydrogen sulfide was very much greater than the drift that was noted with the extract which was used to determine reduced ascorbic acid. These differences may be noted in Table VII.

TABLE VII

Difference in the Readings Taken at 15 Seconds and 30 Seconds on the Spectrophotometer when Determining Ascorbic Acid

Food	Before treatment with H_2S	After treatment with H_2S
Apples	0.1	1.98
Cabbage	0.24	1.80
Onions	0.45	1.60
Potatoes	0.16	1.20

There are several possibilities as to why such a large drift occurred in these extracts after treatment with hydrogen sulfide. Perhaps there was not complete driving off of the hydrogen sulfide by the nitrogen bubbling through the extract, or perhaps some changes occurred in the plant tissue extract to cause this increased reducing action.

Ascorbic Acid Values of the Foods: The values obtained for the reduced ascorbic acid content of the foods in this study compared favorably with the values for these foods that have been reported in the literature, according to Booher, Hartzler and Hewston, 1942. The average amount for the Northern Spy apple in this study was approximately 11 milligrams, which was the same as was reported in the literature for the Northern Spy apple with peeling on, reported on the basis of milligrams per 100 grams moist weight. The average value for the reduced ascorbic acid content of the Danish Ballhead cabbage in this study at the beginning of the storage period was approximately 49 milligrams per 100 grams moist weight. This falls within the range of values reported for Danish Ballhead cabbage in the literature. There was no information available in the literature as to the actual ascorbic acid content of Yellow Globe onions, however, the value of approximately 6 milligrams per 100 grams moist weight, was found to be within the range for onions that has been published. The Chippewa potatoes in this study showed a reduced ascorbic acid content similar to that found to be present in Chippewa potatoes grown in Massachusetts and New York. The amount present at the beginning of the storage period was approximately 11 milligrams per 100 grams moist weight.

SUMMARY AND CONCLUSIONS

1. The ascorbic acid content of Michigan-grown Northern Spy apples, Danish Ballhead cabbage, Yellow Globe onions and Chippewa potatoes during a period of winter storage by two different methods was determined.
2. The Northern Spy apples stored during this study contained approximately 11 milligrams of reduced ascorbic acid per 100 grams of moist material. The Northern Spy apples lost no ascorbic acid during a period of winter storage in a refrigerator or in a home basement.
3. The Danish Ballhead cabbage analyzed in this study contained approximately 49 milligrams of reduced ascorbic acid per 100 grams of moist material at the beginning of the storage period and about 27 milligrams after a storage period of four months in a refrigerator. The cabbage lost ascorbic acid during the period of winter storage and the warmer the storage conditions, the more rapid the loss. Home-stored cabbage showed a greater reduction in ascorbic acid than did the refrigerator-stored cabbage stored the same length of time.
4. The ascorbic acid content of Michigan-grown Yellow Globe onions was approximately 6 milligrams per 100

grams of moist material. There was no significant difference in the reduced ascorbic acid content of the onions when analyzed in the fall and in the spring. There were changes, however, between the fall and spring periods of analyses. There was a decrease in the reduced ascorbic acid content during the first part of the storage period, but this loss did not continue. The home-stored onions showed a significant increase in their reduced ascorbic acid content after an initial period of one month during which there was a significant loss. The trend in the ascorbic acid content of the refrigerator-stored onions also showed an increase after the initial loss shown during the first two months of the storage period.

5. The Chippewa potatoes stored in this experiment contained approximately 11 milligrams of reduced ascorbic acid at the beginning of the storage period and approximately 4 milligrams per 100 grams moist weight at the end of the storage period. The potatoes lost a significant amount of reduced ascorbic acid during a period of winter storage. Potatoes stored in the refrigerator lost ascorbic acid less rapidly than the potatoes that were home-stored.

6. Refrigerator-stored Northern Spy apples and Danish Ballhead cabbage were edible for a period of four

months during the winter while the home-stored apples and cabbage were edible for only two months of the storage period which began in December.

7. Yellow Globe onions and Chippewa potatoes were edible for the entire period of the study whether they were refrigerator-stored or home-stored.

8. Chippewa potatoes and Danish Ballhead cabbage contained significant quantities of dehydroascorbic acid after a period of winter storage.

9. The moisture content of Northern Spy apples and Yellow Globe onions did not decrease during a period of winter storage of four months.

10. The moisture content of refrigerator-stored Chippewa potatoes and Danish Ballhead cabbage did not change to any great extent during the winter storage period. There was, however, a decrease in the moisture content of these two vegetables when they were stored in the home basement.

11. The ascorbic acid content of the cabbage was the highest of the foods studied. Apples were the next best source with onions third on the list and potatoes the last at the end of the storage period.

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