DIURNAL AND SEASONAL CHANGES IN THE ASCORBIC ACID CONTENT OF SOME VEGETABLES

HANS PLATENIUS

(with three figures)

Introduction

As part of a general study concerned with improving the food value of crop plants, a number of experiments have been conducted to determine the factors which control the ascorbic acid content of plants. The most important papers on this subject have been reviewed by Reid (6) and more recently by Maynard and Beeson (3). In spite of some inconsistencies in their results, these investigations lead to the following general conclusions:

1. Mineral nutrition has little influence on the ascorbic acid content of plants except under conditions where normal growth is seriously disturbed by deficiencies in one or more essential elements.

2. Genetic factors play an important rôle. Different varieties of the same species frequently show large differences in the quantity of vitamin C they contain.

3. Some data indicate that the growing temperature affects the rate at which ascorbic acid accumulates in the plant. Moldtman (4) and Reid (5) observed that at moderate temperatures an inverse relation exists between the ascorbic acid content of plants and the temperature at which they are grown.

4. Light, its intensity and the duration of illumination, are known to have a definite influence on the vitamin C content of plant tissue. Reid (6) studied the effect of various light intensities on cowpea seedlings. Sugawara (8) exposed etiolated seedlings of several kinds of vegetables to artificial light of different intensities. Hamner and Parks (2) determined ascorbic acid in turnip greens grown for seven days at light intensities ranging from 200 to 5000 foot candles. All these studies show that, other conditions remaining unchanged, the ascorbic acid of plant tissue increases with rising intensities of light. The magnitude of the increase depends on the duration of the light treatment, the range of intensity studied, and the type of plant tissue involved.

A question of practical importance is whether an appreciable decrease occurs in the ascorbic acid content of crop plants at night. Moldtman (4) observed diurnal fluctuations in the vitamin C content of leaves of deciduous trees which in some instances varied as much as 50 per cent. However, since Moldtman expressed his data as percentages of fresh weight, part of the observed fluctuations were probably caused by corresponding diurnal changes in the water content of the leaves. Reid (6) working with seedlings of cowpea plants found a significant rise in the absolute quantity of vitamin

1 Paper no. 264 Department of Vegetable Crops, Cornell University, Ithaca, N. Y.
C per plant toward early afternoon. Expressed as percentages of dry weight diurnal fluctuations were less than 10 per cent. and the trend was not always consistent.

All these experiments emphasize the importance of light intensity as a factor controlling the ascorbic acid content of plants. A question which remains to be solved is whether under field conditions diurnal periods of light and darkness or periods of cloudy weather have an effect on ascorbic acid large enough to justify changes in the customary procedures of harvesting crop plants.

Materials and methods

Six different vegetables were used in this study: snap beans (*Phaseolus vulgaris*, var. *humilis*); sprouting broccoli (*Brassica oleracea*, var. *italica*); cauliflower (*Brassica oleracea*, var. *capitata*); kale (*Brassica oleracea*, var. *acephala*); spinach (*Spinacia oleracea*); and Swiss chard (*Beta vulgaris*, var. *cicla*). With the exception of snap beans and Swiss chard these vegetables are good sources of vitamin C. The samples were obtained from healthy, vigorously growing plants. In order to minimize errors due to variability among the individual plants, samples of cauliflower and broccoli were taken on successive days from the same head of a single plant. Of the other vegetables not less than 20 leaves or fruits were harvested from an equal number of plants. To obtain comparable samples of kale, which was sampled repeatedly from the same plants over a long period, only leaves near the growing point, measuring 2 to 3 inches in length, were taken.

Within one-half hour after harvest each composite sample was cut up quickly into small sections, mixed thoroughly, and triplicate portions were weighed out for analysis and determination of dry weight.

The method of analysis for ascorbic acid was essentially the same as that devised by Bessey and King (1). Five- or ten-gram samples were extracted with a mixture of one per cent. normal sulphuric acid and two per cent. metaphosphoric acid. A Waring blender served to macerate the tissue. Titrations were made against dichlorophenol-indophenol which was standardized against pure ascorbic acid.

Dry matter was determined by drying the samples in a ventilated oven at 70° C. for 48 hours.

Temperature records were obtained from thermograph records at a level of two feet above ground. The data for solar radiation were calculated from the records of a pyroheliometer which measured light intensities in terms of gram calories per square centimeter. The instruments for recording temperature and solar radiation were located at a distance of about 3 miles from the place where the vegetables used in this study were grown.

Results

In order to determine diurnal changes in the ascorbic acid content of vegetables, samples were taken late in the afternoon between five and seven
o'clock (standard time) and the following morning between six and seven o'clock. Aside from a few exceptions, which will be noted later, sampling was done on a clear day during which the plants received at least ten hours of full sunlight.

The results of ascorbic acid analyses of four different vegetables harvested in the evening and the following morning are given in table I. Expressed on a fresh weight basis, the data show a small, but consistent decrease in ascorbic acid content during the night.

TABLE I
DIURNAL CHANGES IN ASCORBIC ACID AND DRY MATTER OF VEGETABLES

<table>
<thead>
<tr>
<th>VEGETABLES</th>
<th>TIME OF HARVEST</th>
<th>DRY MATTER</th>
<th>PERCENTAGE OF ASCORBIC ACID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FRESH WT.</td>
</tr>
<tr>
<td>Broccoli</td>
<td>7/15 7 P.M.</td>
<td>14.63</td>
<td>1.485</td>
</tr>
<tr>
<td></td>
<td>7/16 6 A.M.</td>
<td>12.12</td>
<td>1.210</td>
</tr>
<tr>
<td></td>
<td>7/16 6 P.M.</td>
<td>15.16</td>
<td>1.450</td>
</tr>
<tr>
<td></td>
<td>7/17 6 A.M.</td>
<td>13.66</td>
<td>1.340</td>
</tr>
<tr>
<td>Broccoli</td>
<td>8/2 5 P.M.</td>
<td>15.40</td>
<td>1.567</td>
</tr>
<tr>
<td></td>
<td>8/3 6 A.M.</td>
<td>13.14</td>
<td>1.329</td>
</tr>
<tr>
<td>Snap beans</td>
<td>7/18 6 P.M.</td>
<td>8.34</td>
<td>0.290</td>
</tr>
<tr>
<td></td>
<td>7/19 6 A.M.</td>
<td>7.93</td>
<td>0.286</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>7/27 7 P.M.</td>
<td>9.32</td>
<td>0.852</td>
</tr>
<tr>
<td></td>
<td>7/28 7 A.M.</td>
<td>8.70</td>
<td>0.737</td>
</tr>
<tr>
<td></td>
<td>7/30 7 P.M.</td>
<td>8.20</td>
<td>0.770</td>
</tr>
<tr>
<td></td>
<td>7/31 7 A.M.</td>
<td>7.77</td>
<td>0.704</td>
</tr>
<tr>
<td>Swiss chard</td>
<td>8/31 5 P.M.</td>
<td>11.26</td>
<td>0.182</td>
</tr>
<tr>
<td></td>
<td>9/1 7 P.M.</td>
<td>9.82</td>
<td>0.178</td>
</tr>
</tbody>
</table>

due partly to slight wilting after exposure to full sunlight and partly to the presence of dew in the morning. After making corrections for these fluctuations in water content by calculating the data for ascorbic acid on a dry weight basis, no consistent differences in vitamin C content could be detected except for cauliflower. Even in cauliflower the loss in vitamin C during the night was less than eight per cent. It should be noted that the data are expressed on a percentage basis. It is possible that because of growth and translocation the changes in absolute quantities of ascorbic acid per plant were somewhat different from those recorded in table I. Unfortunately, the method of sampling used did not permit the calculation of ascorbic acid values on an absolute basis.

According to observations by Reid (6) leaf tissue responds more readily to changes in light intensity than other parts of the plant. For that reason an experiment was conducted in which the ascorbic acid content of kale leaves was recorded consecutively at 6 A.M. and 6 P.M. for a period of five days. These data, expressed as percentages of dry weight are presented in
figure 1 together with corresponding values of solar radiation and temperature recorded at two- and three-hour intervals, respectively. Again the results failed to show consistent variations between samples taken in the morning and evening. The slight rise in the level of ascorbic acid beginning September 14, may reflect the result of a continual exposure to bright sunlight for three days. It is doubtful, however, whether the small difference was larger than the experimental error.

A similar experiment, carried out with spinach, afforded a good opportunity to study the relation between changing light intensities and the ascorbic acid content of the leaves. The results are shown in figure 2. The weather was clear during the first and third day, partly cloudy during the second and fourth, and it rained all day on the fifth day. At the same time the average daily temperature dropped about 23° F. during this period. The concentration of ascorbic acid declined slightly on August 24, the second day of the experiment, rose again the following day, and remained at a uniform level until the end of the experiment. A comparison of these data with corresponding values of light intensity may lead one to attribute the decline in ascorbic acid on August 24 to the fact that it was partly cloudy during that day. However, three days later, when on August 27, solar radiation was at an extremely low level, ascorbic acid values remained practically the same as on the preceding day, thus making it extremely doubtful.
whether a single cloudy day has a depressing effect on the ascorbic content of spinach leaves. It is not impossible, however, that the relatively low temperature prevailing August 27 completely compensated for any depressing effect that the low light intensity may have had.

Although the foregoing experiments make it appear unlikely that single days of cloudy weather have an appreciable effect on the ascorbic acid of leafy vegetables, the possibility exists that differences in light intensities maintained over longer periods of time bring about such an effect. In order to clarify this question, the ascorbic acid content of kale leaves was determined eight different times during the period from July to December. The results may be influenced to some extent by the fact that the plants, from which the samples were taken, increased about three-fold in size during this period. To eliminate this age factor as far as possible, only young leaves of approximately the same size were harvested. All samples were taken at 6 A.M. Weekly averages were calculated for temperature and total daily radiation. The data from ascorbic acid analysis were expressed as percentages of dry matter.

A general downward trend in vitamin C values throughout the season is apparent; it follows the same general trend as the decline in solar radiation (fig. 3). The abrupt drop in light intensity during the middle of October was followed a week later by a sudden decline in ascorbic acid content. A positive correlation between light intensity and concentration of ascorbic acid in the leaves was to be expected on the basis of earlier investigations.
What proved to be surprising was the fact that the total decline in vitamin C between July and October was only about 20 per cent. in spite of the fact that the total solar radiation decreased about 76 per cent. during this period. Perhaps the corresponding drop in average temperature from 72° to 41° F. was sufficient to counteract in part the effect of declining solar radiation.

Although the samples consisted of young leaves of approximately the same size, it is possible that part of the seasonal decline in ascorbic acid of kale can be attributed to the advancing stages in the maturity of the plants. If such a correlation exists it would tend to minimize still further the effect of decreasing light intensities.

Discussion

Based on earlier investigations concerning the effect of light intensity on the ascorbic acid content of plants the suggestion has been made that crop plants be harvested whenever possible following periods of clear, sunny weather. Some have advocated that the picking of vegetables be delayed until the afternoon hours of the day of harvesting, at which time the plants are supposed to have reached their highest content of vitamin C.
The results of the study described in this paper indicate that under field conditions the effect of variable light conditions on the ascorbic acid content of vegetables is small. No diurnal fluctuations in vitamin C content could be detected and single days of cloudy weather were without effect.

There are several reasons which may explain why the studies of Moldtman (4), Reid (7), and others led to different conclusions. Some of the earlier studies were carried out during the winter in the greenhouse, others were made under artificial light at intensities considerably below those prevailing outdoors. Reid (6) and Hamner and Parks (2) have shown that the response to variations in light intensity decreases as higher levels are approached. Consequently, one can expect only small changes in the ascorbic acid content of plants growing outdoors where on bright summer days the light intensity frequently exceeds 10,000 foot candles. Another factor influencing the magnitude of changes in ascorbic acid is the type of plant material used. Reid (5, 6, 7), Moldtman (4), and Sugawara (8) used seedlings in their studies which, according to Reid (6), respond more readily to changes in light intensity than do the tissues of older plants. Even more important is the fact that plants under field conditions are subject to simultaneous changes in solar radiations and temperature. During the summer months, periods of low light intensity usually coincide with periods of relatively low temperatures, two factors which have opposite effects on the level of vitamin C in plants.

Summary

Diurnal fluctuations of ascorbic acid were studied in six kinds of vegetables: snap beans, sprouting broccoli, cauliflower, kale, spinach, and Swiss chard. Expressed on a fresh-weight basis the results showed that these vegetables when harvested in the late afternoon had a slightly higher content of ascorbic acid than those harvested early in the morning. However, these differences were almost entirely due to diurnal fluctuations in the water content of the plants. Calculated as percentages of dry weight the results failed to show consistent differences between morning and evening samples.

The ascorbic acid content of kale and spinach determined at 6 a.m. and 6 p.m. for a period of five days remained remarkably constant. A single day of cloudy, rainy weather had no depressing effect on the vitamin C content of spinach. The concentration of vitamin C in leaves of kale declined about 20 per cent. from July 29 to October 26. Weekly averages of total daily radiation during this period decreased about 76 per cent. It is suggested that a corresponding drop in the average temperature counteracted in part the effect of lowered light intensity.

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Cornell University
Ithaca, New York
LITERATURE CITED


