Table III. General Base Requirement in the Nonenzymatic Formation of Acetoin Catalyzed by Thiamine at pH 8.4 and 35° C. for 24 Hours

[Pyruvic acid (0.1*M*), acetaldehyde (1.0*M*), thiamine (0.1*M*)]

Base	Thiamine	Acetoin Formed, γ
NaOH	Absent	250
	Present	1,500
$Ba(OH)_2$	Present	850
CH_3CO_2K	Present	4,400
K_2HPO_4	Present	10,000

recent publication (*7*). All the previous workers have run their experiments at pH 8.4 and used only one base, sodium hydroxide. Before the reaction could be accepted as the cause of the observed acetoin increase in heated peas, it was necessary to establish several criteria: general base requirement, appreciable rate of reaction at pH 5 to 7, and formation of acetoin from pyruvic acid at low concentrations.

The data in Table III clearly demonstrate the general base requirement for the thiamine-catalyzed formation of acetoin from pyruvic acid and acetaldehyde. The reaction is also influenced by the ionic strength of the solution. The amounts of potassium acetate and dipotassium hydrogen phosphate required to adjust the pH to 8.4 caused a marked increase in acetoin.

Figure 1 shows the data for the amount of acetoin formed at normal vegetable pH; the reaction proceeds at moderate rate under these conditions.

Table IV. Formation of Acetoin in Dilute Pyruvic Acid ($1 \times 10^{-4}M$) and of Thiamine ($1.5 \times 10^{-5}M$) Solutions at pH 7.16

Time of Reaction, Hours	Temp., ° C.	Acetoin Formed, γ
0.0	25	2.6
0.5	100	7.3
1.0	100	6.0
24	25	3.0
48	25	3.0
216	25	3.5

The last question which needed to be answered was the formation of acetoin at the low concentration levels found in vegetables. The data in Table IV illustrate the results of this study at reported thiamine levels in peas and at reasonable pyruvic acid concentration. Very small amounts of acetoin are formed at room temperature even over long periods of time. Significant amounts of acetoin are formed by short heating at 100° C. Thiamine activity is rapidly lost in aqueous solutions at pH 7. Therefore, the 100° C. reaction would soon terminate because of thiamine destruction. Thiamine is much more resistant to heat in vegetable materials than in aqueous solutions (*5*). It would be expected that more acetoin would be formed in the vegetable during heating than is formed in aqueous solutions. The extent of formation would be limited by the amount of pyruvic acid available for reaction.

These studies demonstrate that acetoin can be formed in vegetable products by a nonenzymatic reaction from pyruvic acid catalyzed by thiamine. The results make it desirable to consider the changes of acetoin content during processing, in order to preserve the normal contribution of this important factor to vegetable flavor.

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VEGETABLE VITAMINS

Loss of Vitamin C in Fresh Vegetables as Related to Wilting and Temperature

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Fruits and vegetables furnish approximately 94% of the vitamin C in the diet of urban families in the United States. These foods are largely consumed fresh; temperature and humidity are primary factors in their preservation. Kale, collards, turnip greens, spinach, rape, cabbage, and snap beans were subjected to slow, moderate, and rapid wilting at 32°, 50°, 70°, or 75° F. Conditions favorable to wilting resulted in a more rapid loss of vitamin C. Vegetables that lose moisture readily and wilt appreciably tend to be affected more by humidity and to lose vitamin C more rapidly than those resistant to wilting. Even those that wilt most readily are affected much less by humidity than by temperature.

IF THE RECOMMENDED DAILY dietary allowances of the Food and Nutrition Board, National Research Council, 1953, are accepted as representing the relative amounts required, then vitamin C (ascorbic acid) is required in the diet in greater amounts than all the other vitamins combined. It is notably labile and more readily lost than other food constituents. Vitamin C ingested in excess of the body requirements is not stored in the tissues but excreted, largely as such, in the urine. Consequently, a regular supply must be available if deficiencies are to be prevented.

Fruits and vegetables, including potatoes, furnish 94% of the vitamin C in the diet of urban families in the United States (*2*). These crops are often grown in districts far removed from the

population centers. Even those grown relatively near by may lose much of their vitamin C, unless precautions are taken to preserve it.

Temperature and humidity are major factors in the preservation of fresh fruits and vegetables. Low temperatures retard normal metabolic changes and the development of pathogenic organisms. Low humidity and/or rapid air movement often result in rapid wilting and make the produce less attractive. Also, wilting plasmolysis might hasten oxidation of the cell constituents and result in an adverse effect on the vitamin content.

The literature on this subject is rather limited, inconclusive, and somewhat contradictory (*1*, *3*, *5–7*, *9*). The present studies were undertaken to obtain additional information on the effects of wilting and of temperature on the loss of vitamin C in vegetables.

Materials and Methods

The green leafy vegetables, as a class, are relatively rich in vitamin C, and wilt readily, and were therefore used chiefly in these studies. The vegetables were selected at the local wholesale market. Each test included sufficient material for 3 degrees of wilting at a single temperature. Wet towels spread over the tissue reduced wilting during the period of preparation. Tissues which were as nearly uniform and as representative as feasible were selected. Very immature, old, diseased, and injured leaves, and the outer wrapper leaves from the heads of cabbage were discarded. Because ascorbic acid is unequally distributed and certain plant parts are resistant to wilting, the leaf petioles and midribs, the core from the heads of cabbage, and the tips of the bean pods were also discarded. The leafy tissue was then sliced into strips 2 to 3 cm. wide, and the bean pods were cut crosswise at the center.

The prepared material was mixed, weighed into 50-gram samples, and placed loosely in small, perforated, kraft paper bags. As the bags were filled they were alternately placed so as to form four sublots. Three of the sublots contained 40 replicates each, and the fourth had 10 replicates. The ascorbic acid contents of the replicates in the fourth sublot were determined at the end of the preparation period. The average of these 10 replicates was taken as the initial ascorbic acid content.

The three larger sublots were placed in a single room, thermostatically controlled, at the desired temperature. One sublot (slow wilting) was placed in a metal container over water, covered with wet cloths, and dry cheesecloth was drawn over the top of the counter. A second sublot (moderate wilting) was placed on a wire screen through which air, usually of about 75% relative humidity, could circulate slowly. The third sublot (rapid wilting) was placed on a wire screen within an air chute through which a fan blew air continuously. The bags containing the samples of the moderate- and rapid-wilting sublots were set upright and open to the air. Samples of 10 replicates, comparable with the 10 analyzed at the beginning, were taken at intervals from the different sublots, reweighed to determine loss of weight (degree of wilting) and analyzed for ascorbic acid. Loss of weight, which includes loss of solids used in respiration as well as loss of moisture, is not strictly synonymous with wilting. Nevertheless, it closely approximates the loss of moisture in green leafy vegetables and is used in this paper as a measure of wilting.

The temperatures used were 32°, 50°, and 70°, or 75° F. For each test, at a single temperature, comparable material was used under all humidity conditions. Tests at different temperatures were started on different days, and while these lots of material might contain different amounts of ascorbic acid, at the beginning, the results are comparable as they are reported as per cent lost rather than actual amounts.

Although as nearly uniform conditions as feasible were provided within a treatment, there were appreciable differences in the amounts of weight lost by replicate samples under moderate- and rapid-wilting conditions. Unequal air movement, through the bags, was perhaps the primary case. A piece of tissue strategically placed at right angles to the normal air flow might well affect the rate of drying of an appreciable part of the sample. The variation in wilting, coupled with the normal variation in ascorbic acid, made rather large differences necessary for statistical significance.

Ascorbic acid was determined by the method of Loeffler and Ponting (*8*)

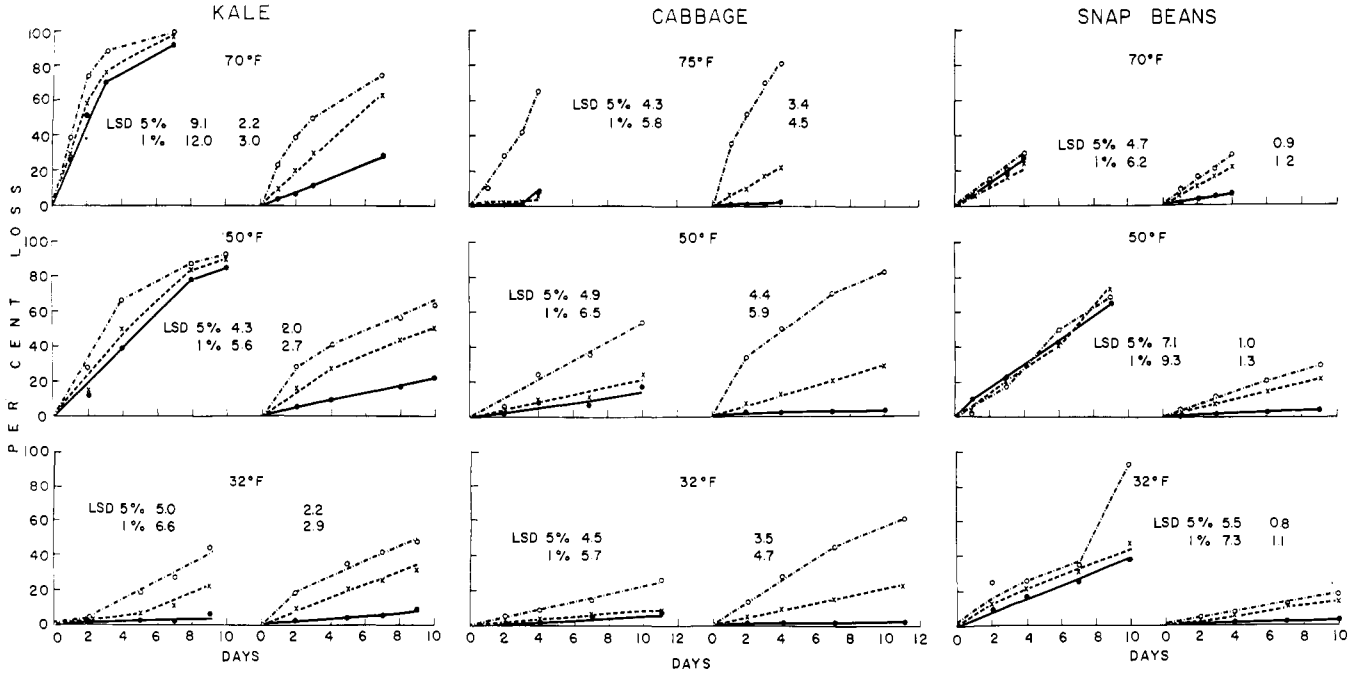

Figure 1. Loss of ascorbic acid as related to wilting and temperature

Left. Loss of ascorbic acid
Right. Loss of weight (degree of wilting)
–.–.–.–.– Rapid wilting
– – – – – Moderate wilting
——— Slow wilting

Ascorbic acid, mg. per 100 grams at beginning of test
Kale, 180, 159, and 159 at 70°, 50°, and 32° F., respectively
Cabbage, 46, 49, and 38 at 75°, 50°, and 32° F., respectively
Snap beans 23, 15, and 20 at 70°, 50°, and 32° F, respectively

Table I. Relative Effects of Temperature and of Wilting on Loss of Ascorbic Acid in Kale during First 2 Days in Storage

Rate of Wilting	Average Loss per Hour,[a] %		
	32° F.	50° F.	70° F.
Slow	0.05	0.32	1.27
Moderate	0.08	0.33	1.45
Rapid	0.11	0.69	1.85

[a] Percentage of ascorbic acid present at time tissue was placed under experimental conditions, calculated for 40-hour storage period.

Each of the 50-gram replicates was extracted with 350 ml. of 0.4% oxalic acid plus 1 ml. of water for each gram of weight lost during the storage period. The 10 replicates were averaged and the ascorbic acid is reported as the percentage lost since the test began. Analysis of variance was used to determine differences required for significance.

Results and Discussion

The effects of wilting and of temperature on the loss of ascorbic acid are shown in Figure 1. Wilting hastened the loss of ascorbic acid in kale, and the differences are statistically highly significant at each of the three temperatures tested. Spinach, collards, turnip greens, and rape were similarly affected and gave curve patterns much like the pattern for kale.

For cabbage the pattern is somewhat different. Normally cabbage loses both ascorbic acid and moisture less rapidly than most leafy vegetables, but when the leaves were separated and the conditions were favorable for wilting, the loss of moisture was fairly rapid and significant differences between treatments were observed. Loss of ascorbic acid was relatively slow, and there was little difference in the rate of loss between the slight-wilting and the moderate-wilting lots. Only in rapid-wilting lots did loss of moisture greatly increase the rate of loss of ascorbic acid.

Snap beans lost moisture rather slowly, and wilting conditions had relatively little effect on the rate of loss of ascorbic acid. Only at 32° F. were there consistent and significant differences in ascorbic acid contents of lots stored under the different wilting conditions; here the differences may have been indirectly influenced by chilling injury, which occurs when snap beans are stored at this temperature.

Wilting thus tends to increase the rate of loss of ascorbic acid in leafy vegetables, but it is of much less importance than unfavorable temperatures (Table I). With kale an increase from little or no visible wilting to a 28% average loss in weight within 2 days resulted in an average increase in loss of ascorbic acid of 60% (from 0.92 to 1.48 mg. per 100 grams per hour). But an increase in temperature from 32° to 70° F. resulted in an average increase from 0.12 to 2.60 mg. per 100 grams per hour, or over 21 times as much. On this basis and with slight to moderate wilting, kale would lose about 40% of its ascorbic acid content if held at 32° F. for 3 weeks, at 50° F. for 4 days, or at 70° F. for 1 day.

Humidity conditions are of greater importance in the preservation of ascorbic acid in vegetables subject to rapid loss of moisture and visible wilting than in those relatively resistant to wilting. As shown in Figure 1, the ascorbic acid content of snap beans was much less affected by humidity conditions than that of green leafy vegetables such as kale. As reported earlier (*4*) humidity conditions caused significant differences in the loss of weight in sweet potatoes, but had little direct effect on the loss of ascorbic acid.

Even though low temperatures are conducive to the preservation of vitamin C in most products, temperatures sufficiently low to cause chilling injury in susceptible products should be avoided as harmful, not only to the product, but also to the vitamin C content.

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ANIMAL GROWTH PROMOTERS

Metabolism of Tritium-Labeled Diethylstilbestrol by Steers

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THE DISCOVERY that adding stilbestrol (α, α′-diethylstilbenediol) to the feed of fattening cattle improves the rate of gain and feed efficiency (*7*) has led to its widespread use in cattle rations. While methods of adapting stilbestrol feeding to various cattle programs have been studied in a multitude of feeding trials, a lack of fundamental knowledge concerning the metabolism of stilbestrol by cattle has made the interpretation of some experimental results difficult.

Considerations of human health have made it necessary to determine whether significant amounts of estrogenic residues are present in the meat of stilbestrol-fed animals. The usefulness of conventional chemical assays in the study of stilbestrol metabolism by ruminants under practical conditions is limited, because of the very small quantities of the estrogen normally administered. However, the high estrogenic activity of stilbestrol has been utilized in biological assays involving in most instances a measurement of the response in weight of the mouse uterus. Bioassays offer a sensitive means of detecting estrogenic activity, but are subject to the effect of other compounds in the tissues which may enhance or depress the response. In the present study the metabolism of stilbestrol by the steer and the occurrence of tissue residues have been studied by tritium labeling. The use of radioactive stilbestrol provides a sensitive tool for the estimation of tissue levels, rates of excretion, and detection of metabolites.

Experimental Procedure

Animals. The experimental animals were two 800-pound yearling Hereford steers which had been fed ground shelled corn, soybean oil meal, and alfalfa hay