Loss of Carotene in Fresh Vegetables as Related to Wilting and Temperature

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Carotene supplies approximately three fifths of the vitamin A values in the normal diet. Dark green, leafy vegetables are especially rich in carotene, but may lose it rapidly unless properly handled after harvest. Temperature and humidity are primary factors in the preservation of these crops. Kale, collards, turnip greens, and rape were subjected to slow, moderate, and rapid wilting at 32°, 50°, and 70° F. Conditions favorable to wilting resulted in a more rapid loss of carotene, but unfavorable temperatures hastened its destruction much more rapidly than did wilting.

Vitamin A is not found in plants, but carotene—provitamin A—occurs regularly in many fruits and vegetables. The structure of β-carotene, the principal carotene of most plants, is such that on hydrolysis in the animal body two molecules of vitamin A may be formed from each molecule of β-carotene. The Food and Nutrition Board, National Research Council (*9*), estimates that carotene and related compounds supply two thirds of the vitamin A activity in the American diet. Leafy, dark green, and deep yellow vegetables are especially rich in these substances and are primary sources of supply for vitamin A values.

Most fruits and vegetables are grown in districts far removed from the population centers, and must be transported long distances to reach the consumer. Conditions surrounding the products during the period between harvest and the time they reach the consumer determine to a large degree what part of the at-harvest vitamin values are available to the consumer.

The major factors in the preservation of fresh vegetables are temperature and humidity. Low temperatures retard normal metabolic changes and the development of pathogenic organisms. Low humidity and/or rapid air movement often result in wilting and a less attractive product. Wilting plasmolysis may also hasten oxidation of cell constituents and thereby have an adverse effect on carotene content.

The literature on the preservation of carotene in leafy vegetables is limited, both as to the effects of temperature and of humidity. With alfalfa, Mitchell and Hauge (*8*) reported that under field conditions little loss of carotene occurs until wilting takes place. Harris and Mosher (*7*), reporting on carotene in lettuce, concluded that the evidence indicates relative humidity—or better its corollary, relative aridity—is at least as important as temperature in the preservation of foods. Ezell and Wilcox (*3*) found that temperature was much more important than humidity, in the preservation of ascorbic acid (vitamin C) in various leafy vegetables, although both affected the rate of loss. They also studied the effect of temperature on the carotene content of sweet potatoes (*4*) and found that while one variety (Nancy Hall) lost carotene during storage at all temperatures, the three other varieties studied tended to lose carotene at unfavorable storage temperatures, but to maintain or increase carotene as more favorable temperatures were provided. Storage humidity (*5*) had relatively little effect on the carotene content of this crop, although loss of moisture in roots stored at low humidities, and/or with rapid air movement, resulted in some shrinkage and weight loss. The resulting concentration of the carotene caused by this shrinkage tended to give the illusion of greater amounts at low humidities. Other workers (*2, 10*) have reported increases in the carotene content of carrots during storage. Similar increases in leafy vegetables have not been established. The present studies were undertaken to obtain additional information on the effects of wilting and of temperature on the behavior of carotene in leafy vegetables.

Materials and Methods

Preparation of the green leafy vegetables used in this study was similar to that described previously (*3*). Kale, collards, turnip greens, and rape were selected at the local wholesale market. Each test included sufficient material for three degrees of wilting at one 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 were discarded. Sheets, Leonard, and Gieger (*11*) reported that the leaf blade contained from 96 to 99% of the carotene in the entire leaf; that the carotene was from 5 to 20 times more concentrated in the blade than in the petiole; and that the carotene was only slightly less concentrated in the petiole than in the midrib. Griffith and Thompson (*6*) reported that carotene was much more stable in alfalfa leaves than in the stems. Leaf petioles and midribs are also less susceptible to wilting than the leaf blade. To increase the homogeneity of the experimental material, the leaf petioles and midribs were removed and discarded. The remaining tissue was then sliced into strips 2 to 3 cm. wide.

The sliced material was mixed, weighed into 50-gram samples, and placed in open, paraffined cardboard containers, perforated on the sides and bottom. As the containers were filled, they were alternately placed so as to form four sublots. Three of the sublots contained 40 replicates each, and the fourth 10 replicates. The carotene 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 carotene 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 container. 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 in a metal container containing calcium chloride, and the top of the container covered with multiple layers of dry cheesecloth. A small fan, with provisions for controlling the speed, was enclosed in this container to stir the

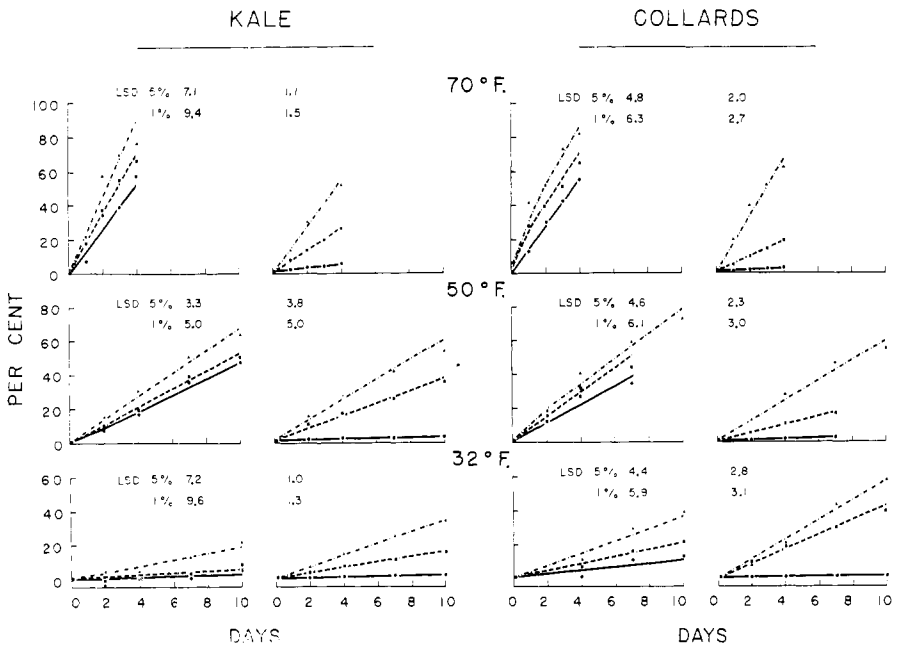

Figure 1. Loss of carotene as related to wilting and temperature

Left. Loss of carotene
Rapid wilting —·—·
Modern wilting – – – –
Right. Loss of weight (degree of wilting)
Slow wilting————

Carotene: Milligrams per 100 grams at beginning of test
Kale: 6.3, 8.9, and 8.2 at 70°, 50°, and 32° F., respectively
Collards: 6.7, 6.1, and 6.1 at 70°, 50°, and 32° F., respectively

Table I. Relative Effects of Temperature and of Wilting on Loss of Carotene during First 4 Days of Storage

Rate of Wilting	Loss of Carotene, %			
	32° F.	50° F.	70° F.	Average
		Kale		
Slow	0.0	17.1	58.5	25.2
Moderate	0.0	18.9	65.9	28.3
Rapid	4.9	31.2	75.8	37.3
Av.	1.6	22.4	66.7	
		Collards		
Slow	2.4	29.5	57.1	29.7
Moderate	7.5	32.4	64.2	34.7
Rapid	12.6	40.9	82.3	45.3
Av.	7.5	34.3	67.9	

air slowly and thus ensure more uniform wilting of the replicates. 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 carotene. 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° 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 initial amounts of carotene, 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 individual samples was perhaps the primary cause.

Carotene was determined as described by Bickoff (*1*). Prior to analysis, the tissue in each replicate was chopped, well mixed, and one fifth of the weight, representing 10 grams of original sample, was transferred to a Waring Blendor and disintegrated at high speed for 1 minute in 100 ml. of acetone. Water, equal to the weight lost during the test period, was added to the wilted tissue to be extracted. A closely-fitted baffle within the blender bowl was used to reduce splashing and avoid loss of solvent during blending. A 5-ml. aliquot of the acetone extract was added to 5 ml. of hexane plus 3 ml. of water in a 50-ml. separatory funnel. The lower aqueous-acetone layer was drawn off and discarded. The entire hyperphase was chromatographed and the carotene content read in a photoelectric colorimeter with a 440-mμ. filter. Results from the 10 replicates were averaged and the carotene is reported as per cent lost since the test began. Analysis of variance was used to determine difference required for significance.

Results and Discussion

The effects of wilting and of temperature on the loss of carotene in kale and in collards are shown (Figure 1). Wilting hastened the loss of carotene in kale, and the difference between rapid and moderate wilting is statistically highly significant at all temperatures. The difference between the slow- and moderate, wilting lots is less than between rapid and moderate, and are of doubtful significance at 50° F. and of no significance at 32° F., where the difference in amounts of wilting was appreciably less than at the other temperatures.

Turnip greens and rape also were studied. The initial carotene content ranged from 4.6 to 5.5 mg. per 100 grams in turnip greens, and 5.8 to 7.3 mg. in rape. Wilting and high temperatures hastened the loss of carotene, and gave curve patterns much like the pattern for kale. At 70° F., significant differences in loss of carotene occurred in both crops under all wilting conditions, but at the lower temperatures, where the rate of loss was slower, the different wilting treatments did not always show statistical significance within the test period.

With collards, the results show highly significant differences at all temperatures, and for all wilting treatments. Humidity differences between slow and moderate wilting were conducive to greater differences in wilting, and greater differences in carotene losses were also recorded. At the lower temperatures, collards lost carotene more rapidly than did kale or any of the other vegetables tested. It was the only one that lost statistically significant amounts of carotene within 4 days at 32° F. under any of the wilting treatments. However, at 70° F. there was little difference in the rate of loss among these crops; kale, collards, and turnip greens lost 58, 57, and 57%, respectively, of their carotene content when held for 4 days at a relative humidity approaching saturation. Excess decay within 4 days prevented collection of comparable data for rape.

Both wilting and unfavorable temperatures hasten the loss of carotene, but unfavorable temperatures are by far the more destructive of the two (Table I). Increased losses of carotene resulting from increased wilting are adequately expressed as a few percentage points over the lower wilting rate. But increased losses resulting from higher temperatures, such as may be encountered in the marketing channels, may better be indicated as a multiple of that lost at the lower temperature.

In a single test, kale was separated into leaves full grown; leaves half- to full-grown; and leaves less than half-grown. All lots were then placed at 32° F. with high humidity. Little or no visible wilting was evident within the 10-day test period. The amount of carotene was greater in the more mature leaves, but the rate of loss was not appreciably affected by the state of maturity.

From the data presented it may be concluded that kale held under conditions that prevent appreciable wilting will lose about one fourth of its carotene content if held at 32° F. for 4 weeks, at 50° F. for 5 days, or at 70° F. for 1 day. If appreciable wilting occurs, these losses may be expected to increase up to 30% at 32° F. and 30 to 40% at 50° and 70° F. Similar results may be expected with turnip greens and rape, and somewhat greater losses with collards. Prepackaging in plastic films effectively reduces the loss of moisture and preserves a fresh, crisp appearance, but low temperatures are also necessary to preserve the vitamin A values normally present in the product.

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Received for review May 2, 1961. Accepted June 29, 1961

A REVIEW OF CONTROL OF MILK COMPOSITION

The Importance of Rumen Metabolism in Relation to Milk Composition

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The substrates utilized by the mammary gland for synthetic and energy purposes in producing milk must be derived from the materials eaten by the cow. The feed constituents undergo extensive degradation and alteration by the bacteria and protozoa present in the rumen. The bovine derives the major portion of its nutrients from the digestion of these microorganisms as well as from the absorption of the products of microbial metabolism. A general discussion of the interrelated metabolism of the rumen microorganisms and bovine organism in regards to protein, carbohydrate, and lipide metabolism is presented. The major purpose of this discussion is to point out the dependence of bovine milk secretion on rumen activity, and to stress that the evaluation of any feeding regime must consider the reactions which occur in the rumen.

THE SUBSTRATES utilized by the mammary gland for synthetic and energy purposes in producing milk must be derived from materials eaten by the cow. The feed constituents enter the rumen where they undergo extensive degradation and alteration by the bacteria and protozoa present. The location of this fermentation vat at the beginning of the digestive tract enables ruminants to make extensive use of materials which would otherwise be of little value. The bovine derives the major portion of its nutrients from the digestion of these microorganisms, as well as from the absorption of the products of microbial metabolism. Because of this, the effects of a particular feed on the metabolism of the bovine must always be evaluated with regards to the influence it exerts on the rumen microorganisms. By selection of certain microorganisms, or by stimulating them to form adaptive enzymes, the products of rumen fermentation can be altered. A change in microbial end products available to the host animal can possibly have an effect on the metabolism of the cow and the composition of milk.

The study of rumen function is complicated by the dynamic nature of the system where production, interaction, and absorption of metabolites are continuously occurring. The frequent intake of food by the animal provides a regular supply of substrates for the microorganisms. At the same time, the soluble products of microbial activity appear to be readily absorbed through the rumen wall. Likewise, the volume of rumen contents is regularly influenced by two factors. Large volumes of saliva secreted by the host animal are continuously being added to the rumen contents, while at frequent intervals, a portion of the rumen material (small food particles and microorganisms) passes to the omasum and the lower digestive tract. The concentration of a metabolite in the rumen would then be determined by the interplay of all these factors. The study of the effect of a dietary component, mediated by the rumen, on the metabolism of the cow is hindered by the fact that the cow is in a sense eating all the time. Changes in the level of metabolites in the blood after a