same investigators (*24*). Results in this laboratory (*5*) show that at least 30% of the total sulfur of such methanolic extracts can be accounted for as cycloalliin. Table III shows that the total amount of these three amino acids raises the value to the range found by the other methods. Furthermore, Virtanen and Matikkala (*26*) have reported the presence of sulfur-containing γ-glutamyl peptides, which could conceivably give rise enzymatically to pyruvic acid, possibly from noncyclic precursors to cycloalliin. The amount of pyruvic acid developed in garlic macerates (*1, 9*) averages about five times that found in onions.

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VEGETABLE DETERIORATION

N^6-Benzyladenine, a Senescence Inhibitor for Green Vegetables

F. W. ZINK

University of California, Davis, Calif.

Green vegetables deteriorate steadily and rapidly after harvest. Protein content declines, and chlorophyll content decreases in close proportion. The loss of quality inevitable in such produce is minimized by rapid, careful handling and the best possible storage conditions. Kinetin-like chemicals delay senescence of green vegetables and offer an additional means of reducing quality losses in perishable crops. N^6-benzyladenine applied to green vegetables delays visual manifestations of senescence that occur during storage. This senescence inhibitor was effective in maintaining the green color and appearance of lettuce, endive, escarole, Brussels sprouts, sprouting broccoli, mustard greens, radish tops, celery, parsley, green onions, and asparagus.

GREEN VEGETABLES deteriorate steadily and rapidly after harvest. Protein content declines, and chlorophyll content decreases in close proportion, as shown by detached-leaf studies (*2–4*). Loss of quality is minimized by rapid, careful handling and good storage conditions. However, the finding by van Overbeek (*6*) that kinetin-like chemicals delay senescence of green vegetables offers an additional means of reducing quality losses in these perishable crops.

This paper reports the results of a study of the effect of N^6-benzyladenine (known experimentally as SD 4901) on the rate of deterioration of several green vegetables, with stress on response rather than on the biochemical modes of action.

A series of experiments was conducted in 1958–1960 to test the effect of dosage, methods, and time of application of N^6-benzyladenine on the rate of deterioration of several vegetables. Two types of holding tests were run: those in which the vegetables were harvested, trimmed, packed as gently as possible, and held at 40° or 70° F.; and a series with vegetables held 8 days at 40° and then transferred to 70° F., thus simulating an 8-day transit period from California to New York, and a following period in retail outlets. All vegetables were harvested from commercial fields in the central coastal district of California. Each treatment consisted of ten matched heads, stalks, buds, etc., depending on the vegetable. Samples were at market

Table I. Effect of Date of Postharvest Application of N^6-Benzyladenine on Marketability of Great Lakes Lettuce

Pretreatment Storage Conditions	% of Sample Marketable after Indicated Day at 70° F.											
	0	1	2	3	4	5	6	7	8	9	10	11	12
2 days at 40° F.													
Control	100	100	90	90	90	90	80	30	0				
Treated[a]	100	100	100	100	90	90	90	70	50	20	10	0	
4 days at 40° F.													
Control	100	..	90	80	70	70	40	20	10	0			
Treated[a]	100	100	100	100	100	100	90	80	70	40	20	10	0
6 days at 40° F.													
Control	100	100	100	100	100	70	50	30	10	0			
Treated[a]	100	100	100	100	90	80	80	70	60	40	30	0	
8 days at 40° F.													
Control	100	100	100	80	70	40	20	10	0				
Treated[a]	100	100	100	100	90	90	70	60	60	20	10	10	0

[a] Treatment at rate of 5 p.p.m. N^6-benzyladenine to runoff point. All heads trimmed to 4 wrapper leaves at harvest.

maturity and free of visible diseases and physical defects.

A quality score was used in which a rating of 9 = freshly harvested, 7 = good, with minor defects, 5 = fair, with few defects, and 3 = unsalable. The samples were examined periodically, a quality rating was given each specimen in the sample, and the percentage of the sample that was salable was recorded. The curves are drawn as the best fit to the average of all sample ratings.

Effect on Leafy Vegetables

Lettuce treated with N^6-benzyladenine remained fresh and green longer than the control. Figure 1 shows the results of a simulated transit test with the head lettuce variety Great Lakes. These results are typical of the response of head lettuce treated with 5 p.p.m. in 100 gallons per acre just before harvest.

A series of postharvest treatments was made on head lettuce held 2, 4, 6, and 8 days at 40° F., treated with a 5-p.p.m. spray to runoff stage, then transferred to 70° F. for holding. Results in Table I suggest possible use of this senescence inhibitor as a postharvest treatment at the terminal market.

Treatment increased the storage life of leaf and butterhead lettuce varieties, both relatively poor shippers that deteriorate more rapidly than head lettuce. Figure 2 shows the response of Big Boston to a 5-p.p.m. application in 100 gallons per acre at harvest in a simulated transit and holding test. A similar response was observed with Cos lettuce.

The response of endive, escarole, spinach, and mustard greens to N^6-benzyladenine was striking. Loss of quality in these vegetables is characterized by a rapid disappearance of chlorophyll from the older leaves, and infection by fungi and bacterial soft rots. Table II reports the percentage of heads salable in a holding test with escarole. The results are typical of the response for this group of leafy vegetables.

Carrots and radish are often marketed

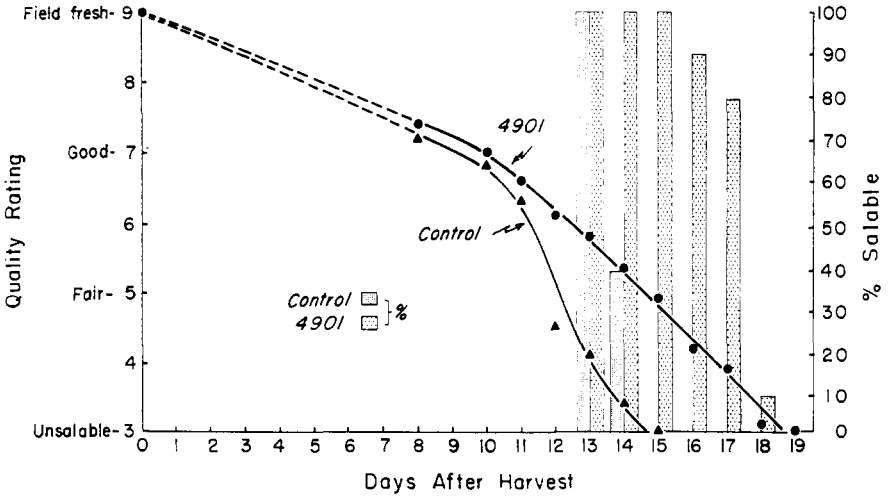

Figure 1. Effect of day of harvest treatment with N^6-benzyladenine on quality rating and percentage of sample salable

Great Lakes lettuce held 8 days at 40° F., then transferred to 70° F.

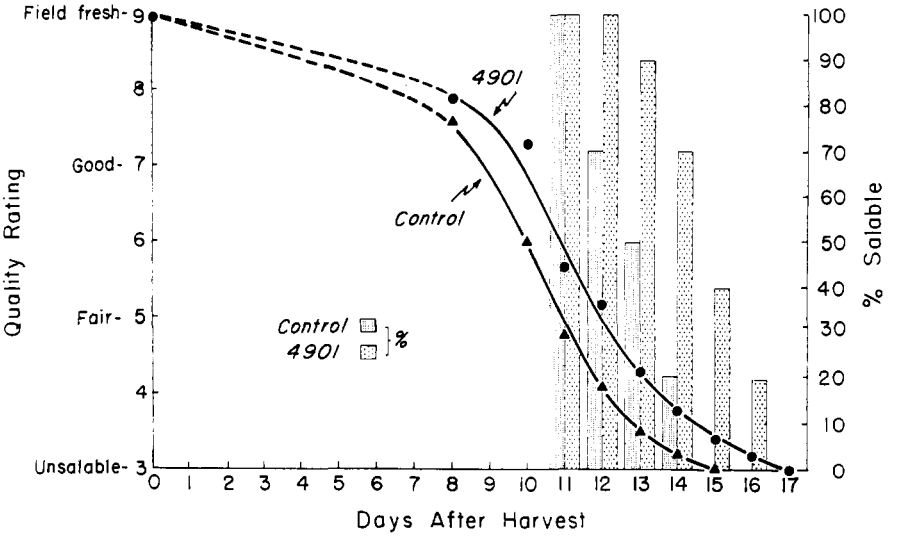

Figure 2. Effect of day of harvest treatment with N^6-benzyladenine on quality rating and percentage of sample salable

Big Boston lettuce held 8 days at 40° F., then transferred to 70° F.

with tops attached to indicate freshness. N^6-Benzyladenine extended the storage life of these tops. The tests indicate that treated tops will have a quality rating of 5 (fair) or above for about 2 to 3 days longer than untreated tops.

Effect on Nonleafy Vegetables

N^6-Benzyladenine extended the storage life of several vegetables whose buds, stalks, or spears are marketed. Brussels sprouts retained a green color and bright appearance longer if treated (Figure 3).

Table II. Effect of Day of Harvest Treatment with N^6-Benzyladenine on Marketability of Escarole Held at 70° F. after Harvest

Treatment[a]	% of Sample Marketable on Indicated Day after Harvest							
	0	3	4	5	6	7	8	9
Control	100	80	60	30	0			
5 p.p.m. N^6-benzyladenine	100	90	70	60	60	40	20	0

[a] Sprayed to runoff point.

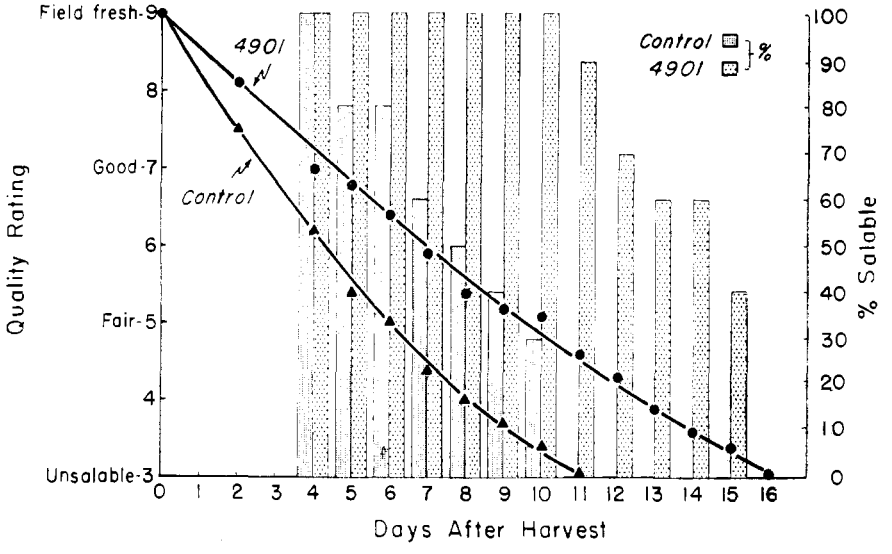

Figure 3. Effect of day of harvest treatment with N^6-benzyladenine on quality rating and percentage of sample salable

Brussels sprouts held at 70° F. after harvest

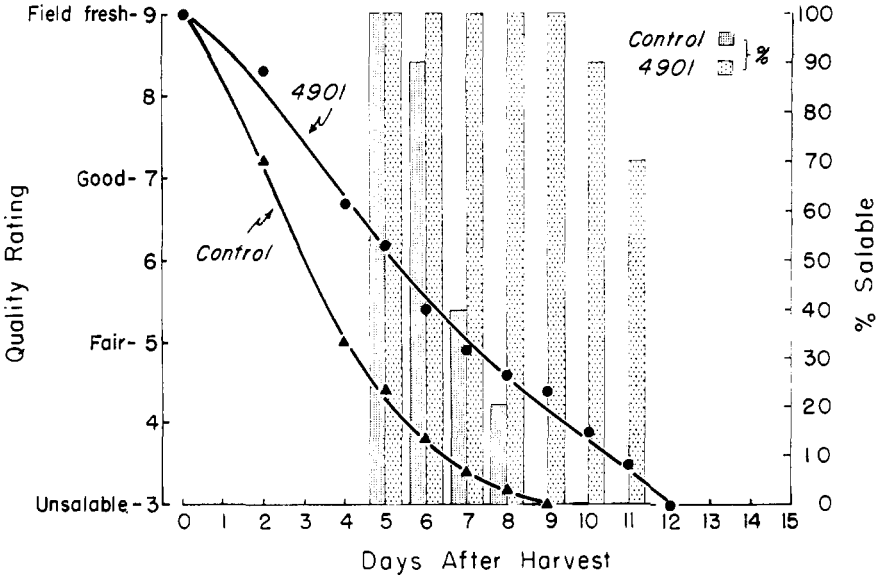

Figure 4. Effect of day of harvest treatment with N^6-benzyladenine on quality rating and percentage of sample salable

Green bunching onions held at 70° F. after harvest

Chlorophyll disappeared rapidly from the outer leaves of untreated sprouts and they became dull and flaccid. Preliminary studies indicate that the storage life of sprouting broccoli, celery, asparagus, and green onions can be extended by N^6-benzyladenine applied at harvest. Figure 4 shows a typical response of green bunching onions to a dip treatment of 5 p.p.m. at harvest.

Methods, Rates, and Timing of Applications

Solutions of N^6-benzyladenine can be applied as field sprays to field-packed crops such as lettuce. The optimum dosage per acre varies somewhat with different crops, depending on the density of the foliage. Thorough coverage is necessary; 2.5 to 10 p.p.m. at 75 to 150 gallons per acre should be used. Great Lakes lettuce responded well to 5 and 10 p.p.m. solutions at 100 gallons per acre. Bessey (*1*) obtained similar results in Arizona. Results were best with applications as near harvest as possible. With lettuce, applications on the day of harvest gave the most satisfactory response, and applications made more than 3 to 4 days before harvest were essentially ineffective. These results suggest that the effect of N^6-benzyladenine disappears rapidly under field conditions.

Vegetables handled through packing sheds can be treated with dips and washes. The concentrations found best were in the range of 2.5 to 10 p.p.m. Dipping and washing time does not appear to be critical, though thorough coverage is necessary. Higher dosages (20 p.p.m.) may cause phytotoxicity, as evidenced by marginal necrosis and wilting. In the crops studied there appears to be a severalfold margin of safety between optimum and phytotoxic concentrations.

N^6-Benzyladenine will not improve the appearance of produce that already shows moderate to severe symptoms of senescence. Postharvest applications to produce rated at less than 5 (fair) are ineffective in slowing deterioration. Response has been favorable with lettuce, carrot tops, parsley, and celery held 8 days at 40° F. before treatment.

This senescence inhibitor was effective in maintaining the green color and appearance of lettuce, endive, escarole, Brussels sprouts, sprouting broccoli, leaves on cauliflower, mustard greens, radish tops, carrot tops, celery, parsley, green onions, and asparagus. No response was observed in artichokes, snap beans, or Lima beans.

The results show that N^6-benzyladenine applied to green vegetables delays yellowing and other visual manifestations of senescence that occur during transit and storage. A common result of senescence in vegetables is the storage

rots caused by bacteria and fungi growing on amino acids and other nutritive substances leaking out of aging cells. N^6-Benzyladenine appears to delay this by maintaining cell vigor. It seems probable that its effect is partially due to a delay in proteolysis. Richmond and Lang (*5*) showed that kinetin can reduce or prevent the accelerated protein loss that is typical of detached leaves; at the same time, it delays the loss of chlorophyll and extends the life span of the leaf. It is thus not a biocide but acts through its effect on the physiology of the tissue. Use of this senescence inhibitor would not be an alternative to present practices, but would be a supplement to rapid, careful handling, and proper storage conditions.

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WHEAT GLUTEN PROTEINS

Amino Acid Composition of Proteins in Wheat Gluten

J. H. WOYCHIK, J. A. BOUNDY, and R. J. DIMLER

Northern Regional Research Laboratory, Peoria, Ill.

The recent chromatographic isolation of purified protein components from wheat gluten resulted in a program of basic studies on the structure of these proteins as related to the unique physicochemical properties of gluten. The first step was determination of the amino acid composition of six gluten components or fractions. While their compositions were similar, significant differences were observed which indicate the individuality of these components. Glutamic acid and proline together accounted for one half or more of the peptide-bound amino acids, and therefore probably are of particular structural importance in the gluten proteins. The composition of a water-soluble fraction isolated from gluten preparations indicated that it corresponds to albumins and/or globulins wheat.

THE UNIQUE physicochemical properties exhibited by hydrated wheat gluten proteins have prompted numerous investigations designed to explain the chemical and physical structures responsible. Particular emphasis has been placed on the role of sulfhydryl and disulfide bonding in gluten proteins (*2, 4, 11*). Although much information has been obtained from these and related studies, the ultimate explanation of gluten behavior will require broader fundamental investigations of gluten protein structure.

The recent development in this laboratory of a chromatographic technique for separating and isolating purified gluten proteins on carboxymethylcellulose columns (*12*) has permitted the initiation of a program of basic studies on gluten protein structure.

The first step was to determine the amino acid composition of purified gluten components using the ion exchange procedure of Moore, Spackman, and Stein (*5*). Six gluten components or fractions were analyzed.

Experimental

Preparation of Gluten Proteins. Proteins corresponding to the electrophoretic components demonstrated in Ponca hard red winter wheat gluten by Jones, Taylor, and Senti (*3*) were studied in this investigation. These components, with the exception of alpha-1, were isolated by chromatographic fractionation on carboxymethylcellulose (*12*). The alpha-1 component was obtained by pH precipitation (*3*).

Amino Acid Analysis. Protein samples (25 mg.) were hydrolyzed with constant boiling hydrochloric acid distilled from an all-glass apparatus. Each sample was dissolved in 12 ml. of acid contained in a 25-ml. borosilicate glass tube. The tubes were sealed and the hydrolysis was carried out in an autoclave held at 110° ± 2° C. for 24 hours. The hydrolyzates were light tan in color and contained very small amounts of humin. After centrifugation, the humin was removed and analyzed for nitrogen; no significant amount of humin nitrogen was found in any hydrolyzate. Hydrochloric acid was removed by repeated evacuation on a rotary evaporator at 40° C. Protein samples for cystine analyses were oxidized with performic acid by the method of Schram and Bigwood (*9*) prior to hydrolysis. Tryptophan content was estimated by the colorimetric procedure of Spies and Chambers (*10*). The nitrogen content of 2-ml. aliquots was determined by duplicate micro-Kjeldahl analyses.

The preparation and use of Amberlite IR-120 columns were as described by Moore, Spackman, and Stein (*5*), except that the basic column was increased in length to 25 cm. and eluted at pH 5.28 with 0.33*N* instead of 0.35*N* buffer. These modifications were necessary to prevent overlapping of histidine and ammonia peaks as a result of the high level of amide ammonia in the hydrolyzates. Effluent fractions of 2 ml. were collected by drop count with a Technicon automatic fraction collector. Amino