J. AMER. SOC. HORT. SCI. 112(5):794–797. 1987.

Vitamins C, B_1, and B_2 Contents of Stored Fruits and Vegetables as Determined by High Performance Liquid Chromatography

Alley E. Watada
Horticultural Crops Quality Laboratory, Agricultural Research Service, U.S. Department of Agriculture, BARC-West, Beltsville, MD 20705

Tony T. Tran
Department of Horticulture, University of Maryland, College Park, MD 20741

Reprinted from Vol 112(5), September 1987
Journal of the American Society for Horticultural Science
Alexandria, VA 22314

Additional index words. ascorbic acid, thiamine, riboflavin, snap beans, cauliflower, squash, sweet potatoes, strawberries, potatoes, temperature

Abstract. **A high performance liquid chromatography (HPLC) method was developed for analyzing vitamin B_1, and the method was used with other HPLC methods to monitor vitamins C, B_1, and B_2 of fresh fruits and vegetables stored at different temperatures. Ascorbic acid content of most commodities either dropped after being placed in storage or decreased during storage. Thiamine increased during storage of 'Tendergreen' beans and decreased in 'BelRus' potatoes; whereas riboflavin decreased in 'Tendergreen' beans and increased in 'Superior' potatoes. The changes in other cultivars or commodities were not significant.**

Considerable data are available on the vitamin C (ascorbic acid) content of selected stored fresh fruits and vegetables (3, 5, 7); whereas the data on vitamins B_1 (thiamine) and B_2 (riboflavin) of similar commodities are very limited (17). Ascorbic acid probably was analyzed preferentially because the quantity in 100 g of many commodities is sufficient to satisfy much or more of the U.S. recommended daily allowance (USRDA), whereas the quantity of thiamine and riboflavin in 100 g will satisfy only $\leq$10% of the USRDA.

Accuracy and precision of vitamin analyses have improved significantly with the use of HPLC. Several HPLC procedures have been reported for analyzing vitamins (8, 12–14), but most are applicable only to samples such as vitamin tablets or fortified food in which the quantity of each vitamin is equivalent to about 100% of the USRDA. In fruits and vegetables, the content differs widely among vitamins, and the low content of B vitamins requires sensitive methods for analysis. Effective and sensitive HPLC methods have been developed for ascorbic acid (15) and riboflavin in horticultural crops (16). A sensitive HPLC method for analyzing thiamine in meat and meat products (1) was found to be unsatisfactory for fresh fruits and vegetables in this study.

This study was undertaken to develop an HPLC method for thiamine analysis and to monitor the contents of ascorbic acid, thiamine, and riboflavin of fresh fruits and vegetables that were stored at different temperatures.

Materials and Methods

Fresh fruit and vegetables were obtained from growers in Delaware, Maryland, and Pennsylvania, and were prepared for the study either on the day of harvest or after holding overnight at 5°C. Samples were free of defects, uniform in size, and were separated into sublots to have three replicates of each temperature–time regime treatment indicated under Results and Discussion. With some commodities, ascorbic acid, thiamine, and riboflavin were analyzed from each sublot; whereas with other commodities thiamin and riboflavin were analyzed from one sublot, and another sublot was used for ascorbic acid. Ascorbic acid was analyzed on the day of removal from storage. Samples for thiamine and riboflavin were weighed, frozen in liquid nitrogen, freeze-dried, and kept at −70° until analysis.

The cultivar, month, year of harvest, and source are as indicated in Table 1.

Analytical procedures. Ascorbic acid and riboflavin were extracted and analyzed by HPLC as described elsewhere (15, 16).

Thiamine: Sample preparation. A 0.5-g, freeze-dried, ground sample was mixed with 50 ml of 0.1 N HCl, placed in a circulating water bath at 95°C for 30 min, cooled to room temperature, neutralized with 5 ml of 2 N NaOAc, treated with 5 ml of 5% mylase (w/v), and incubated overnight at 38°. The enzyme reaction was terminated with the addition of 2 ml of 50% trichloroacetic acid (w/v) and the mixture was placed in a 60° water bath for 5 min. The mixture then was cooled, pH adjusted to 4.00 with 4 N NaOAc, and water added to bring the volume up to 100 ml. The mixture then was filtered through a Whatman no. 40 filter. Five milliliters of 0.04% potassium ferricyanide was added to a 5-ml aliquot of the filtrate to oxidize thiamine to thiochrome, followed by addition of 13 ml of isobutanol, which formed a nonaqueous phase containing the thiochrome. This layer was filtered through a Durapore membrane disk filter with a 0.22-μm pores.

HPLC. The thiochrome was separated in a Waters μPorasil 3.9 × 30 cm column with a 4 × 25 mm guard column containing Waters Type II Corasil. The Corasil was deactivated before use by washing with 100 ml of water and was replaced after 200 injections. The mobile phase consisting of 2% isobutanol, 38% methanol, and 60% chloroform (all from Burdick and Jackson) was pumped at a rate of 1 ml·min^{-1} with Waters 6000A solvent delivery system. Thiochrome was quantified with a Perkin–Elmer fluorescence spectrophotometer, Model 650-105, with the excitation wavelength at 362 nm and the emission wavelength at 420 nm.

Received for publication 10 Sept. 1986. We thank Kathryn Hoff for the technical assistance and Robin Cohen for the statistical analysis. Use of a company or product name by the USDA does not imply approval or recommendation of the product to the exclusion of others which may also be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 1. Cultivar, month, year of harvest, and source of commodities that were stored and analyzed for vitamins.

Commodity	Genus and species	Cultivar	Harvest month and year	Source
Beans, snap, green	*Phaseolus vulgaris*	Tenderpod	Aug. 1982	Clinton, Md.
Beans, snap, green	*Phaseolus vulgaris*	Tendergreen	Aug. 1982	Clinton, Md.
Cauliflower	*Brassica oleracea* var. botrytis	Snowcrop	Oct. 1982	Preston, Md.
Squash, summer yellow	*Cucumis pepo*	Multipik	July 1982	Salisbury, Md.
Squash, summer yellow	*Cucumis pepo*	Multipik	Aug. 1982	Clinton, Md.
Sweet potatoes	*Ipomea batatas*	Centennial	Sept. 1983	Salisbury, Md.
Strawberries	*Fragaria ×ananassa*	Red Chief	June 1983	College Park, Md.
Strawberries	*Fragaria ×ananassa*	Early Glow	June 1983	College Park, Md.
Potatoes	*Solanum tuberosum*	BelRus	Nov. 1982	Orono, Maine
Potatoes	*Solanum tuberosum*	Superior	Nov. 1982	Orono, Maine

Table 2. Concentration and percentage recovery of thiamine in fruits and vegetables when extracted and analyzed as described in the text and content as reported in the USDA Agriculture Handbook 8-11 (6).

Commodity	Thiamine (μg/100 g fresh wt) HPLC	AH 8-11	Percent recovery
Apples, Golden Delicious	16	17	86
Beans, snap, green	132	85	97
Cauliflower	73	76	90
Pepper, sweet, green	36	85	96
Spinach	76	78	105
Squash, summer, yellow	65	70	98
Sweet potato	62	60	97
Potatoes, russett	68	88	85

Table 3. Contents of vitamins C, B_1, and B_2 in 100 g fresh weight of several crops at harvest, as measured by HPLC methods.

Crop and cultivar	Vitamins C (mg)	B_1 (μg)	B_2 (μg)
Green beans, Tenderpod	16.0 ± 1.3[z]	---	---
Green beans, Tendergreen	24.9 ± 1.7	132 ± 3	120 ± 2
Squash, yellow, Multipik			
Salisbury, Md.	23.4 ± 0.6	---	---
Clinton, Md.	21.4 ± 1.5	55 ± 5	45 ± 2
Strawberry, Early Glow	77.7 ± 1.0	---	---
Strawberry, Red Chief	48.0 ± 0.5	22 ± 3	9 ± 1
Potato, BelRus	20.1 ± 0.8	51 ± 1	23 ± 1
Potato, Superior	27.4 ± 0.2	68 ± 3	19 ± 2
Cauliflower	71.0 ± 5	---	---
Sweet potatoes	27.7 ± 1.0	56 ± 5	31 ± 4

[z]Mean ± SE. Each mean includes three replicates.

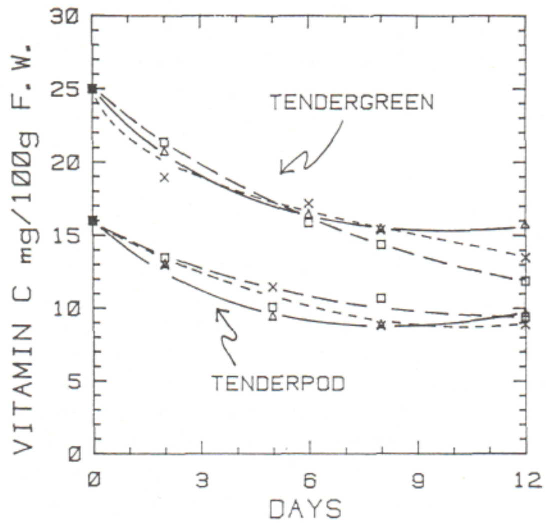

Fig. 1. Vitamin C content of 'Tendergreen' and 'Tenderpod' beans held at 5° (△), 10° (★), and 15°C (□). Each point represents the average of three samples.

Results and Discussion

HPLC method for thiamine. The procedures used for acid hydrolysis and enzyme hydrolysis to extract thiamine had an effect on the data. Acid hydrolysis at 90° to 95°C in a water bath resulted in repeatable results with maximum values, whereas the AOAC procedure of hydrolysis in an autoclave, 121° and 103 kPa (2), resulted in inconsistent data. Enzyme hydrolysis was required to cleave the phosphate esters and proteins from the thiamine. Mylase was as effective as takadiastase. The latter is no longer available. A series of mylase concentrations ranging from 1% to 10% were evaluated for effectiveness, and 5% (w/v) was found to be the minimum concentration required. Addition of papain with mylase resulted in inconsistent data, so papain was excluded.

A sufficient quantity of potassium ferricyanide is required for a complete conversion of thiamine to thiochrome; however, an excessive quantity can cause destruction of thiochrome (11). For the amount of thiamine in fruits and vegetables, a 0.04% concentration of potassium ferricyanide was adequate and a 0.08% concentration was not excessive.

Table 4. Regressions for contents of vitamins C, B_1, and B_2 in 100 g fresh weight of green beans, summer squash, strawberries, cauliflower, and potatoes on storage time (days) and temperature (°C).

Crop (cultivar)	Regression equation[z]	Percentage of total sums of squares[y] Variable (%) 1st	2nd	3rd	R^2
Green beans (Tenderpod)	Vitamin C = 15.9 − 1.4 days + 0.08 days^2	68	17	---	0.86
Green beans (Tendergreen)	Vitamin C = 23.6 − 1.6 days + 0.06 days^2	72	5	---	0.77
Squash[x] (Multipik)[w]	Vitamin C = 27.5 − 1.94°C + 0.08°C^2	28	26	---	0.56
Strawberry (Early glow)	Vitamin C = 63.5 + 1.3°C − 0.95 days − 0.05°C^2	49	8	31	0.88
Cauliflower[x] (Snowcrop)	Vitamin C = 77.8 − 5.1°C + 0.26°C^2	5	69	---	0.75
Green beans (Tendergreen)	Vitamin B_1 = 121 + 2.24°C + 0.82 days − 0.08°C^2	19	13	16	0.48
Potatoes[x] (BelRus)	Vitamin B_1 = 81.4 − 0.122 days	51	---	---	0.51
Green beans (Tendergreen)	Vitamin B_2 = 12.4 − 4.82 days + 0.29 days^2	8	32	---	0.41
Squash[x] (Multipik)[w]	Vitamin B_2 = 49.4 + 0.67°C − 0.05°C^2	23	21	---	0.44
Potatoes[x] (BelRvs)	Vitamin B_2 = 23.79 + 0.036 days	23	---	---	0.60
Potatoes[x] (Superior)	Vitamin B_2 = 23.4 + 0.075 days	56	---	---	0.56

[z]Regressions are applicable only to time and temperature described in text.
[y]Percentage of total sums of squares partitioned to each variable when entered in the order given.
[x]Data from initial analysis (0 day) were not included in the regression.
[w]Squash from Clinton, Md.

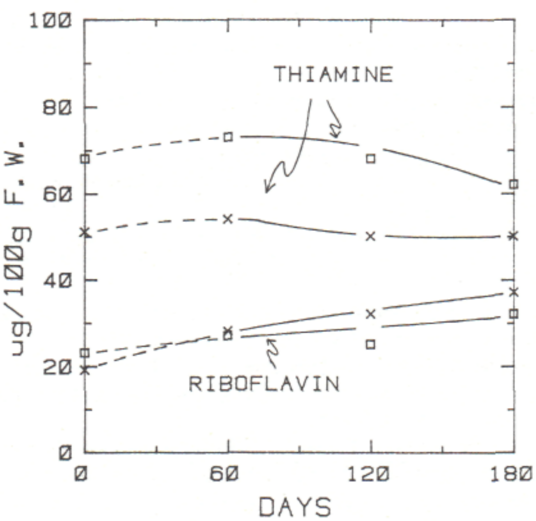

Fig. 2. Thiamine (B_1) and riboflavin (B_2) contents of 'BelRus' (□) and 'Superior' (x) potatoes held at 3°C. Each point represents the average of three samples.

Elution time decreased with increased methanol content in the mobile phase and was 3.7 min with 38% methanol, 2% isobutanol, and 60% chloroform delivered at a rate of 1 ml·min^{-1}. The 2% isobutanol was necessary to buffer against polarity and viscosity changes that occurred with the injection of isobutanol sample mixture in an isobutanol-free mobile phase. Without the isobutanol in the mobile phase, the chromatogram peak of thiochrome was slightly broad and tailed. When solvent of a brand other than the original was used, elution time changed and the chromatogram peak was not sharp. This change was resolved by addition of 1% EDTA in the mobile phase as suggested by R. Gourly (personal communication). We speculate that minute amounts of minerals were present in the solvent or were released from the HPLC by the solvents.

This HPLC procedure is sensitive to very low levels of thiamine, and responds linearly for levels ranging from 0 to 1.5 ng. A sensitive method described recently by Fellman et al. (4) for measuring thiamine and riboflavin simultaneously was not evaluated for comparative purpose.

Recovery of added thiamine ranged from 85% to 105% with several fruits and vegetables (Table 2). Recovery was good for green beans, bell peppers, spinach, summer yellow squash, and sweet potatoes, whereas it was low for apples, cauliflower, and russet potatoes. The cause for low recovery is not known.

Values for thiamine content of five of the eight commodities reported here were similar to those reported in the USDA Agriculture Handbook 8–11 (6) (Table 2). For the remaining three commodities, values were larger for green beans and smaller for green peppers and russet potatoes than the handbook values. The differences probably are due to differences in cultivars and analytical procedures, which are not described in the USDA handbook.

Vitamin content. Ascorbic acid content of harvested fruits and vegetables differed with cultivar and location of production (Table 3). 'Early Glow' strawberries contained 62% more ascorbic acid content than 'Red Chief'. 'Tendergreen' beans contained

56% more than 'Tenderpod', and 'BelRus' potatoes contained 35% more than 'Superior'. Similar differences among cultivars have been reported for other commodities (7). The effect of location was studied only with the 'Multipik' squash. Those obtained from Salisbury contained more ascorbic acid than those from Clinton, Md. Salisbury is east of the Chesapeake Bay on sandy loam soil, whereas Clinton is west of the Chesapeake Bay and south of Washington, D.C., on sandy to clay loam soil.

Cultivar comparison for thiamine and riboflavin was made only with the potatoes. 'Superior' contained 33% more thiamine than 'BelRus', whereas 'BulRus' contained 21% more riboflavin than 'Superior'.

Changes during storage. Ascorbic acid of green beans decreased during storage and was not affected by temperatures of 5°, 10°, or 15°C during 12-day storage (Fig. 1). Regression equations for both cultivars were similar with most of the changes dependent on the linear component, ≈70% of the total sum of squares (Table 4). The nonlinear component was 5% and 17% of the total sum of squares for 'Tendercrop' and 'Tendergreen', respectively.

Ascorbic acid contents of squash samples decreased by 10% to 15% when initially analyzed after storage, which was the second day, but the content remained unchanged thereafter during the 12-day storage. Storage temperatures affected only squash from Clinton, Md. The regression equation (Table 4) depicts higher values at 5°C than at either 10° or 15°, and the similar values at 10° and 15°.

Time and temperature had an effect on the ascorbic acid content of 'Early Glow' but not 'Red Chief' strawberries. The regression equation for 'Early Glow' strawberries indicates the higher ascorbic acid values at lower temperatures and the values decreasing with time (Table 4). Analyses were terminated after the 3rd, 7th, and 10th day at 20°, 10°, and 0°C, respectively, due to deterioration. Time or temperature did not affect the 'Red Chief' ascorbic acid.

Ascorbic acid of cauliflower did not change during 14 and 30 days storage at 10° and 0°C, respectively. Values at 0° were higher than those at 10°, and the values at 20° were higher than those at 0° or 10° as depicted by the regression equation. Samples at 20° were analyzed only once, the 3rd day after storage, because of deterioration.

Ascorbic acid contents of sweet potatoes and potatoes dropped by 18% and 40% on the 21st and 30th day of storage, respectively, which was the first analysis during storage. Thereafter, contents remained unchanged during the final 63 days of storage of sweet potatoes at 15°C and 120 days storage of potatoes at 3°.

Thiamine contents of only 'Tendergreen' beans and 'BelRus' potatoes were affected by storage. Contents of 'Tendergreen' beans increased during storage with the content and increase being slightly greater at 10°C than at 5° or 15°. The regression equation (Table 4) to predict these changes is limited to beans held 4 days at 15°, 10 days at 10°, and 16 days at 5°. Thiamine in 'BelRus' potatoes decreased after being in storage (Fig. 2) and the regression is described in Table 4. Contents of 'Superior', as reported for 'White Rose' (17), did not change significantly. On the other end of the spectrum, an increase has been noted with 'Triumph' and 'Irish Cobbler' during 24 weeks of storage (10).

Riboflavin of 'Tendergreen' beans decreased sharply during the first few days of storage as depicted by the regression equation (Table 4). This regression is limited to a 12-day storage period. Riboflavin of 'Superior' potatoes increased during the 180-day storage (Fig. 2), whereas the increase of 'BelRus' were not significant. Yamaguchi (17) reported that riboflavin in 'White Rose' potatoes did not change during storage.

The contents of vitamins analyzed in this study did not change consistently among the fresh fruits and vegetables. Others (7) have shown that ascorbic acid generally decreases during storage; however, this decrease was not always the observed in this study. Kopec (9) has indicated that synthesis of ascorbic acid occurs in some commodities, and degradation is noted in others. Results on thiamine and riboflavin indicate that these two B vitamins change minimally during storage, and any changes that occur probably will have a minimal effect on its contribution to the USRDA.

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