# β-Carotene and Ascorbic Acid Retention in Fresh and Processed Vegetables

L.A. Howard, A.D. Wong, A.K. Perry, and B.P. Klein

**ABSTRACT**

**Broccoli, carrots, and green beans (grown in 2 consecutive years) were randomly divided into 3 treatments: fresh-refrigerated (F-R), frozen (FZ) or canned (C) (carrots only). FZ or C vegetables were processed within 24 h and stored for up to 1 yr. F-R vegetables were held at 4 °C for 3 wk (broccoli and green beans) or 6 mo (carrots). Trans β-carotene (Tβ-C) and total ascorbic acid (AA) were determined at specified times, before and after microwave cooking. Vitamin content differed between years due to environmental conditions. Blanching resulted in AA loss, but retention remained stable after freezing broccoli and green beans. F-R green beans lost >90% AA after 16 d storage. Linear decreases in AA were found in most F-R or FZ vegetables. Tβ-C decreased slightly during freezer storage. Reductions in Tβ-C occurred in canned carrots. Microwave cooking had minimal effects on AA or Tβ-C.**

**Key Words: broccoli, carrots, green beans, ascorbic acid, β-carotene**

## INTRODUCTION

THE HEALTH BENEFITS OF VEGETABLES ARE WELL RECOGNIZED by nutritional and medical communities, but intakes in the United States are below recommendations (Block et al., 1992; Subar et al., 1995; Muñoz et al., 1997; Dennison et al., 1998). Published reports link fruit and vegetable intakes with reduced risk of chronic diseases like cardiovascular disease and cancer (Sies and Stahl, 1995; Steinmetz and Potter, 1996; Franceschi et al., 1998). Important components of vegetables include vitamins, particularly those that act as antioxidants. Of the antioxidant compounds, ascorbic acid (AA) and β-carotene are present in the greatest quantity in vegetables. β-carotene has been identified as a potential anticarcinogen (Liebler, 1993; Gaziano et al., 1995; Sies and Stahl, 1995), as well as an antioxidant and vitamin A precursor (Sies and Stahl, 1995).

Changes in vitamin content during postharvest handling, processing and storage have been reported by many, but comparing studies and predicting nutrient retention remain unreliable. As early as 1945, Van Duyne and co-workers established that 15% to 20% of AA was lost during blanching, and AA content gradually decreased during 9-mo frozen storage. Because of its lability, AA is routinely used as an index to measure processing effects on nutrient retention (Erdman and Klein, 1982; Klein and Perry, 1982; Vanderslice et al., 1990).

Studies of β-carotene during storage and processing of vegetables show no definite trend of nutrient retention, but fluctuate among samples analyzed in different laboratories. Reports range from no loss to slight or marked decrease when total carotenoids were measured (McConnell et al., 1945; Martin et al., 1960). Wu et al. (1992) found that β-carotene remained stable in both refrigerated and frozen broccoli during storage. However, AA decreased rapidly in refrigerated green beans after 3 days, but remained stable in refrigerated broccoli. Blanching before freezing resulted in small losses (<10%) of AA in green beans, and larger losses (~40%) in broccoli. Frozen storage resulted in no further losses of AA.

Variations in nutrient content of starting material will influence final vitamin content in processed vegetables. Concentrations can vary depending on vegetable type, maturity at harvest, genetic variations, preharvest conditions, postharvest handling, storage conditions, processing, and preparation (Albrecht et al., 1991; DeRitter, 1982; Klein and Perry, 1982; Selman, 1994; Gregory, 1996). Studies of AA and β-carotene in processed vegetables have been conducted using retail market fresh vegetables of unknown origin that may have been in refrigerated storage for up to 2 wk during transport and distribution. The nutrient content of supermarket fresh vegetables may be lower than that of processed, especially if harvested several days before purchasing (Dietz and Erdman, 1989).

Many unknown factors make it unreliable to compare studies of commercially processed vegetables conducted at different times. Although the same cultivars can be grown for processing and fresh consumption, past studies have not made this direct comparison and no multiyear studies have been reported. Few investigations have been published on the effect of refrigerated storage on nutrient retention in freshly harvested vegetables during the first postharvest week. No studies have compared nutrient content of refrigerated and processed vegetables of the same cultivar.

Our objective was to compare broccoli, green beans, and carrots because they are among the most widely consumed vegetables in the United States and are eaten in fresh and processed forms. Our study was designed to make a controlled examination of the influence of thermal processing (canning or blanching), refrigerated and frozen storage, and microwave cooking on the retention of trans β-carotene and total AA in fresh-refrigerated (F-R), frozen (FZ) or canned vegetables of a single cultivar of broccoli, green beans, and carrots, of known history. Each vegetable was harvested from a single field; broccoli and carrots were grown in 1994 and 1995, and green beans in 1995 only.

## MATERIALS & METHODS

### Experimental design

Three field replications of broccoli and carrots were grown at the Univ. of Illinois Horticulture Research Center in St. Charles, Ill., U.S.A. Broccoli (*Brassica oleraceae* L. var. 'Arcadia') was planted in early August 1994 and late March 1995 and harvested in late October and late July, respectively. Carrots (*Daucus carota* L. var. 'Minicor') were planted in April and harvested in July, respectively. Three field replications of green beans (*Phaseolus vulgaris* L. var. 'Blue Lake') were grown at the Green Giant Agricultural Farm in Manito, Ill., U.S.A. in 1995. Crops were packed in wooden crates and transported in a truck that was cooled to 4 °C with dry ice. Processing took place at the Univ. of Illinois Food Science Pilot Plant in Urbana, Ill., U.S.A. The vegetables were stored 8 to 12 h in a walk-in cooler at 4 °C prior to processing.

Samples were randomly divided to undergo one of 3 treatments: fresh-refrigerated (F-R), frozen (FZ), or canned (C) (carrots only). Washed vegetables (~500 g) in the F-R treatment group were placed

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in Ziploc® vegetable bags (~2.5 microholes/cm$^2$, density 1.75 with vapor transmission 1.3) (Hanlon, 1992) and stored at 4 °C. Broccoli and green beans were stored for 3 wk, and carrots were stored for 6 mo. Broccoli samples were analyzed 1, 3, 7, 14, and 21 d after harvest (DAH) in 1994 and 1, 5, 9, 16, and 21 DAH in 1995. Green beans were analyzed 1, 4, 8, and 16 DAH in 1995. Carrots were analyzed 1, 7, 14, 21, 42, and 84 DAH in 1994 and 1, 5, 15, 20, 35, and 84 DAH in 1995.

Prior to freezing, vegetables were washed, cut, and blanched on a steam conveyor belt. Broccoli florets (~5 cm dia and ~3 cm stalk) were steam blanched for 110 sec (blancher chamber temperature of 90 ± 2 °C). Green bean ends were removed, and the remaining portions were cut into 3-cm pieces and steam blanched at 90 ± 2 °C for 60 sec. Carrot ends were discarded, and the remaining portion was cut in 5- to 7-cm lengths. The carrots were abrasion peeled for 30 sec and then steam blanched for 120 sec (97 ± 2 °C). All vegetables were cooled in ice water for 1.5 min, and excess water was drained. A peroxidase test, using guaiacol as substrate, was conducted to ensure proper blanching (Luh and Woodroof, 1975). Blanched vegetables (454 g) were packaged in heat sealed polyethylene commercial freezer bags and frozen at –40 °C in an air blast freezer. After 2 d, the frozen vegetables were placed in a chest freezer (Kenmore Model 10458) at –20 °C for long term storage. Frozen storage periods for broccoli were 3, 21, 49, 112, 224, and 365 DAH in 1994 and 5, 9, 21, 84, 112, 224, and 365 DAH in 1995. Frozen storage periods for carrots were 3, 28, 56, 112, 224, and 365 DAH in 1994 and 5, 20, 35, 112, 224, and 365 DAH in 1995. Green beans were stored for 4, 8, 21, 112, 224, and 365 DAH in 1995.

Prior to canning, carrots were cut and abrasion peeled (as for frozen samples), water blanched at 74 ± 2 °C for 5 min, and cooled in an ice water bath for 1.5 min. Carrots (250 ± 5 g) were packed into 300 × 407 cans and covered with 190 ± 5 g of hot brine solution (95.22% water, 2.67% sugar, 1.90% salt, 0.13% citric acid monohydrate, and 0.08% calcium chloride dihydrate). Cans were sealed and placed in a Steritort (FMC Corp., San Jose, Calif., U.S.A.). Parameters for canning were: initial temperature 71 °C, process temperature 127 °C; and cool down temperature 38 °C. Cans were stored at 22 °C for 3, 28, 84, 182, and 365 DAH in 1994 and 1, 5, 20, 35, 112, 224, and 365 DAH in 1995.

### Microwave heating preparation

Two bags of F-R whole vegetables were washed, cut, and 454-g portions were removed for microwave cooking and analyses. Each vegetable was cut to the same dimensions as frozen samples. Broccoli and water (40 g) were cooked for 8 min, carrots and water (60 g) for 9 min, and green beans and water (40 g) for 7 min.

Two bags of FZ vegetables were combined and 454-g portions plus 30-g water were cooked for 9 min. Canned carrots (454 g) and brine (60 g) were heated for 3.5 min. All the vegetables were cooked in a 2-L Pyrex® glass covered casserole dish in a Panasonic® microwave oven at full power (700 W). Samples were stirred halfway through cooking time.

### Moisture determination

Duplicate homogenized samples (~3 to 5 g) were placed on aluminum weighing dishes at 55 °C for 24 h, transferred to a vacuum oven (65 °C), and dried for 24 h. Samples were cooled in a desiccator and weighed.

### Ascorbic acid analysis

Extracts were prepared by blending 1% m-$HPO_3$ (100 mL) and vegetable (75 g) in a Waring Blendor for 2 min. The sides of the blendor jar were washed with 1% m-$HPO_3$ (50 mL) and blended for an additional 2 min. The slurry was adjusted to 250 mL with 1% m-$HPO_3$ and then filtered using Whatman 2V fluted filter paper. Vegetable filtrates (broccoli, 1 mL; carrots, 2.5 mL; and green beans, 2.5 mL) and 5% dithiothreitol (0.5 mL) were mixed. Broccoli extracts were diluted to 10 mL with 1% m-$HPO_3$. Extracts were filtered through a 0.20 µm filter, and 10 µL were injected onto the liquid chromatograph.

The HPLC method used is similar to that described by Sapers et al. (1990) with modifications. Total AA concentration was measured using an isocratic HPLC system consisting of a Beckman Model 421 controller, Beckman Model 100A pump, and Beckman Altex C-R1A Integrator with a Waters M-490 Programmable Multiwavelength Detector. The stationary phase was a Rainin Dynamax-60Å amine column (4.6 × 250 mm protected by a Rainin amine guard module (8 µm, 1.5 cm). The mobile phase consisted of acetonitrile: 0.05 M $KH_2PO_4$ (75:25; pH 5.95) at a flow rate of 1.5 mL/min. Detection was at 268 nm with a sensitivity of 0.02 absorbance units full scale (AUFS). AA standards (USPC Inc., Rockville Md., U.S.A.) were prepared in 1% m-$HPO_3$ with 5% dithiothreitol and diluted to 10 ppm AA. Standards were run daily with sample extracts for validation. Values for AA were expressed as mg/100 g vegetable.

### Trans β-carotene analysis

The method used was a modification of the HPLC method described by Dietz et al. (1988). Vegetable extracts were prepared by grinding 100 g for 10 sec in a R2 Ultra Robot-Coupe Food Processor. Ground samples (~3 g) were placed in 70 mL test tubes with 10 mL ethanol (0.l g BHT/100 mL ethanol) and mixed for 30 min using a Tekmar Tissumizer. KOH (1 mL, 100 g KOH/100 mL) was added and mixtures were saponified at 70 °C for 30 min, 2-mL water were added and cooled to room temperature. The aqueous layer was extracted 3 times with 8 mL hexane and solvent was decanted under vacuum into a Büchner funnel with PS1 filter paper. The aqueous layer was discarded, and the extract was diluted to 10 mL (broccoli and green beans) or 25 mL (carrots) with hexane. Samples (5 mL) were filtered through a 0.45 µm filter and 20 µL and injected onto the liquid chromatograph column. The system consisted of a Beckman Model 421 controller, Beckman Model 100A pump, and Beckman Altex C-R1A Integrator with a Waters M-490 Programmable Multiwavelength Detector. A Vydac C18 column (4.6 × 250 mm) was protected by a Vydac C18 guard module (5 µm, with titanium frit). The mobile phase was methanol:acetonitrile: water (88:9:3) at a flow rate of 1.5 mL/min. Absorbance was measured at 457 nm with sensitivity of 0.01 AUFS. β-carotene standards were run daily with the samples. β-carotene content is expressed as IU Vitamin A/ 100 g of vegetable.

### Statistical analyses

The vegetables in each experimental unit (field) were randomly divided into 3 replications, processing method (F-R, FZ, C), and preparation (uncooked, microwave cooked). Data were statistically analyzed by repeated measures analysis of variance (ANOVA) to examine changes in levels of AA or β-carotene over storage periods in the two-year study, using polynomial contrast transformations ($a = 0.05$) (SAS Institute Inc., 1991). To determine the influence of microwave cooking on the concentrations of AA, β-carotene, and moisture, least significant differences (LSD at $\alpha = 0.05$) were used to compare means calculated for total storage time.

## RESULTS

### Broccoli

**Moisture content**. There was no change ($p > 0.05$) in moisture content of F-R broccoli grown in 1994/1995 (87.7 ± 0.2%/ 88.1 ± 0.09%) during the 3-wk storage period (87.8 ± 0.45%/ 89.5 ± 0.5%). Steam blanching prior to freezing and frozen storage resulted in no change ($p > 0.05$) in moisture content in either year. Microwave cooking decreased ($p < 0.05$) moisture content by ~3% in F-R and FZ broccoli in 1994 and 1995 at each storage time.

**Total AA content**. Total AA contents (wet weight basis, WWB) of F-R and FZ uncooked and microwave cooked broccoli for 1994 and 1995 were compared (Figs. 1a and 1b). Mean concentrations of total AA (WWB) in fresh broccoli within 24 h postharvest in 1994

and 1995 were 123.3 ± 7.6 mg/100 g and 179.7 ± 18.1 mg/100 g. AA retention during refrigerated storage exhibited linear decreases in both years ($p < 0.01$; Fig. 1a). Note that in 1995 AA content decreased rapidly in the first 3 DAH, and the average retention was 52% after 3 wk, while in 1994, the average loss of AA after 3 wk was only 13%.

AA content, averaged over the total storage time, for cooked and uncooked broccoli was compared (Table 1). Microwave cooked F-R broccoli had similar AA content to uncooked ($p > 0.05$).

Steam blanching prior to freezing decreased AA concentration ~30% in both 1994 and 1995. AA content was 90.6 ± 8.6 mg/100 g and 135.6 ± 24.5 mg/100 g, respectively, post blanching. After freezing, AA concentration decreased ($p < 0.05$) in both 1994 and 1995. The concentration of AA in FZ steam blanched broccoli after 3 d in 1994 declined 16% (86.4 ± 3.5 mg/100 g) and after 5 d in 1995, 29% (77.2 ± 3.7 mg/100 g). Overall, the patterns of loss in both years were linear ($p < 0.01$) (Fig. 1b). Microwave cooking of FZ steam blanched broccoli resulted in lower AA content ($p < 0.05$) in both years (Table 1).

**Tβ-C content.** Tβ-C concentrations (IU Vitamin A/100 g, WWB) of F-R and FZ uncooked and microwave cooked broccoli in 1994 and 1995 were compared (Figs. 2a and 2b). Initial mean concentrations in F-R broccoli in 1994 and 1995 were 327 ± 55 and 705 ± 104 IU/100 g. Tβ-C concentrations after 3 wk storage increased 19% in 1994 and decreased 64% in 1995. There was no pattern ($p > 0.05$) of retention observed in 1994 or 1995. Microwave cooking of F-R broccoli resulted in higher Tβ-C concentration ($p < 0.05$) in each year (Table 2).

Measurable Tβ-C concentration was 22% higher in blanched broccoli in 1994 (420 ± 13 IU/100 g), but 48% lower in 1995 (365 ± 21 IU/100 g) after blanching. Freezing resulted in a 15.7% decrease of Tβ-C in FZ steam blanched broccoli in 1994 (357 ± 47 IU/100 g) and had no effect on the Tβ-C in 1995 (385 ± 22 IU/100 g) during 1 yr storage. There was a linear decrease ($p < 0.01$) in 1994 and 1995 (Fig. 2b). Microwave cooking of FZ steam blanched broccoli resulted in an increase in Tβ-C concentration ($p < 0.05$) in both years (Table 2).

**Table 1—Mean ascorbic acid concentration (mg/100 g) for total storage time in fresh-refrigerated (F-R), frozen (FZ), and canned (C) uncooked and microwave cooked vegetables**

| Sample Preparation-Yr[c] | Broccoli | Carrots | Green Beans |
|---|---|---|---|
| F-R Uncooked 94 | 114.5[a] | 3.9[a] | |
| F-R Microwave 94 | 116.5[a] | 4.7[a] | |
| F-R Uncooked 95 | 115.9[a] | 4.2[b] | 8.2[a] |
| F-R Microwave 95 | 111.9[a] | 5.5[a] | 9.6[b] |
| FZ Uncooked 94 | 88.6[a] | 4.6[a] | |
| FZ Microwave 94 | 80.2[b] | 3.8[a] | |
| FZ Uncooked 95 | 76.8[a] | 1.9[a] | 13.1[a] |
| FZ Microwave 95 | 69.1[b] | 1.7[a] | 12.1[b] |
| C Uncooked 94 | | 0.6[a] | |
| C Microwave 94 | | 0.8[a] | |
| C Uncooked 95 | | 0.4[b] | |
| C Microwave 95 | | 0.2[a] | |

[a,b]Means followed by the same letter in each group are not significantly different ($p>0.05$).
[c]Groups in the first column are compared for each vegetable. Means were calculated from data at all storage times for cooked or uncooked states (n=15 for F-R broccoli in 1994 and 1995, FZ broccoli in 1994; n=12 for F-R green beans; n=18 for all others).

## Carrots

**Moisture content.** Moisture content of F-R carrots in 1994 and

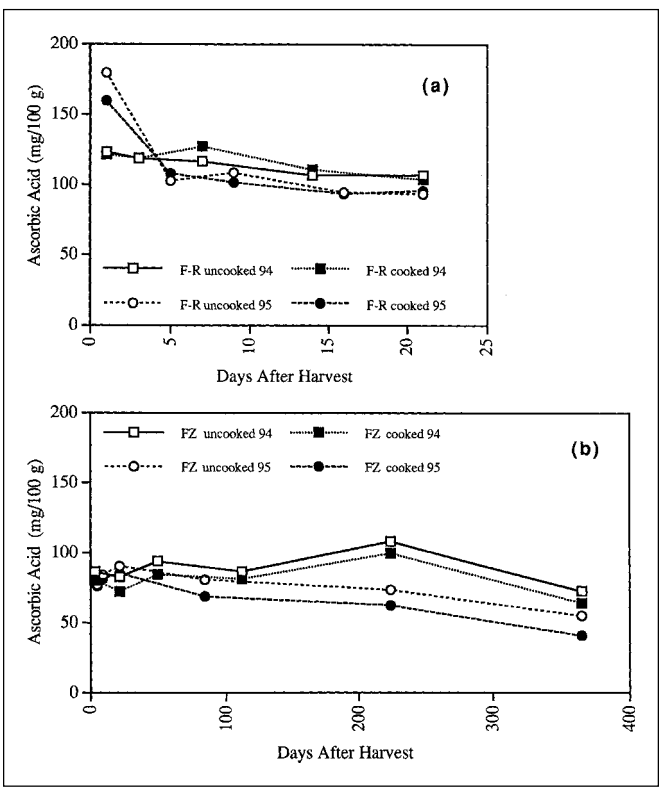


**Fig. 1—Ascorbic acid content in broccoli harvested in 1994 and 1995: (a) fresh-refrigerated (F-R) uncooked stored at 4 °C for 21 d and microwave cooked; (b) frozen (FZ) uncooked stored at –20 °C for 1 yr and microwave cooked.**

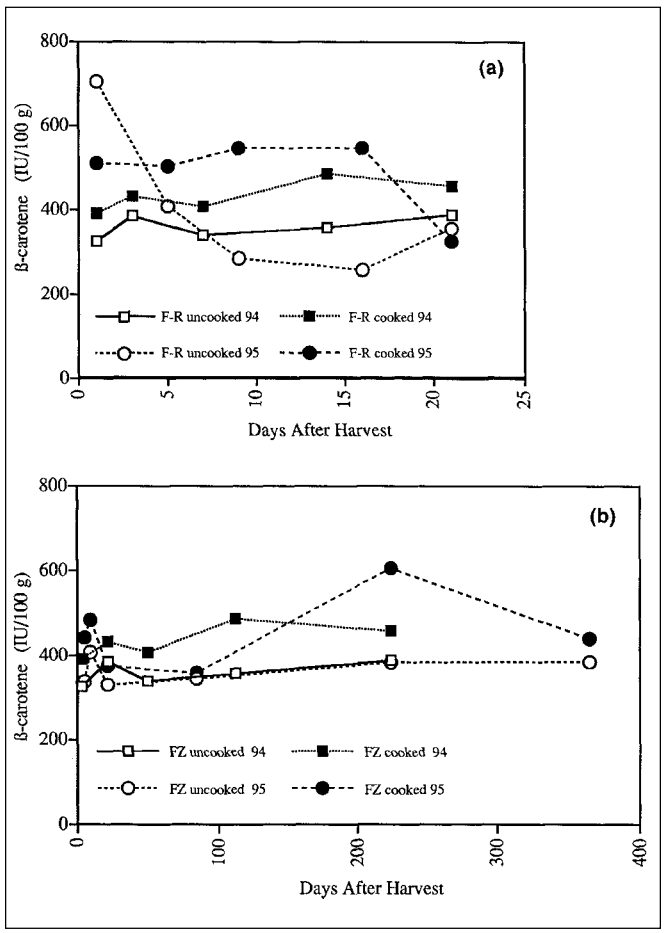


**Fig. 2—Trans β-carotene content in broccoli harvested in 1994 and 1995: (a) fresh-refrigerated (F-R) uncooked stored at 4 °C for 21 d and microwave cooked; (b) frozen (FZ) uncooked stored at –20 °C for 1 yr and microwave cooked.**

**Table 2—Mean β-carotene concentration (IU Vitamin A/100 g) for total storage time in fresh-refrigerated (F-R), frozen (FZ), and canned (C) uncooked and microwave cooked vegetables**

| Sample Preparation-Yr[c] | Broccoli | Carrots | Green Beans |
|---|---|---|---|
| F-R Uncooked 94 | 360.1[b] | 3906.9[a] | |
| F-R Microwave 94 | 435.2[a] | 3817.6[a] | |
| F-R Uncooked 95 | 403.3[b] | 4364.5[a] | 182.6[a] |
| F-R Microwave 95 | 487.4[a] | 4590.0[a] | 155.2[b] |
| FZ Uncooked 94 | 325.8[a] | 2158.4[b] | |
| FZ Microwave 94 | 333.4[a] | 2602.1[a] | |
| FZ Uncooked 95 | 373.6[b] | 2593.5[b] | 192.4[a] |
| FZ Microwave 95 | 446.9[a] | 3141.3[a] | 180.3[a] |
| C Uncooked 94 | | 2059.5[a] | |
| C Microwave 94 | | 1800.5[a] | |
| C Uncooked 95 | | 2762.3[b] | |
| C Microwave 95 | | 3258.1[a] | |

[a,b]Means followed by the same letter in each group are not significantly different (p>0.05).
[c]Groups in the first column are compared for each vegetable. Means were calculated from data at all storage times for cooked or uncooked states (n=15 for F-R broccoli in 1994 and 1995, and FZ broccoli in 1994; n=12 for F-R green beans; n=18 for all others).

1995 (87.9 ± 1.4% and 87.5 ± 0.5 % respectively) did not change ($p > 0.05$) during 84-d storage (87.3 ± 0.2 and 87.9 ± 0.7). No changes ($p > 0.05$) in moisture content occurred as a result of steam blanching prior to freezing, nor during 1 yr FZ storage. Microwave cooking of F-R and FZ carrots decreased moisture content ~3% ($p < 0.05$), but canned carrots were not affected by microwave cooking.

**Total AA content.** AA contents of F-R, FZ, and canned carrots, uncooked and microwave cooked, for 1994 and 1995 were compared (Figs. 3a, 3b, and 3c). Initial concentrations of AA in freshly harvested carrots in 1994 and 1995 were similar (4.3 ± 0.4 mg/100 g and 3.9 ± 0.4 mg/100 g), confirming that carrots are not a good source of this vitamin. After 1-wk storage in 1994, AA concentration in carrots decreased (2.5 ± 0.5 mg/100 g). However, AA content increased ~50% (6.5 ± 0.7 mg/100 g) in 1995. AA concentration (6.8 ± 0.5 mg/100g) in carrots after 14-d storage in 1994 was similar to carrots after 1 wk storage in 1995. AA concentrations in 1994 and 1995 decreased after 3 wk and values were similar (3.4 ± 0.6 mg/100 g and 3.1 ± 0.5 mg/100 g, respectively). AA retention in F-R carrots in both years exhibited no pattern during the 84-d storage (Fig. 3a). When AA was averaged over the total storage time for cooked and uncooked carrots, F-R uncooked carrots contained similar amounts of AA to microwave cooked F-R carrots ($p > 0.05$) (Table 1).

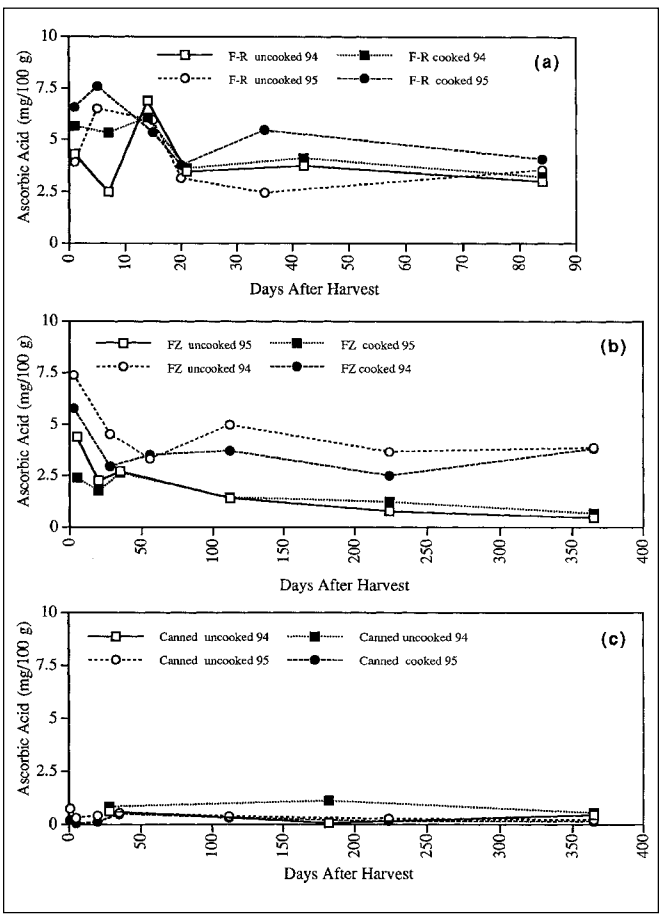


**Fig. 3—Ascorbic acid content in carrots harvested in 1994 and 1995: (a) fresh-refrigerated (F-R) uncooked stored at 4 °C for 84 d and microwave cooked; (b) frozen (FZ) uncooked stored at –20 °C for 1 yr and microwave cooked; and (c) canned stored at 22 °C for 1 yr and microwave cooked.**

Steam blanching of freshly harvested carrots prior to freezing did not affect AA concentration (4.3 ± 0.2 mg/100 g) in 1994, but caused a decrease ($p < 0.05$) (2.8 ± 0.2 mg/100 g) in 1995. AA content of FZ steam blanched uncooked carrots after 3 days storage in 1994 and 1995 was 7.4 ± 1.9 mg/100 g and 4.4 ± 0.5 mg/100 g, respectively (Fig. 3b). After 3 wk, AA concentration decreased in both years. There was no pattern observed in the retention of AA after 1-yr storage at –20 °C in 1994; however, a linear decrease ($p < 0.05$) was exhibited in 1995. AA content of FZ carrots was not affected by microwave cooking (Table 1).

Prior to canning, carrots were hot-water blanched to remove intercellular gas. AA content in carrots after hot water blanching decreased 14% ($p < 0.05$) (Fig. 3c). Canning resulted in a decrease ($p < 0.05$) in AA content in 1994 and 1995. AA concentrations in canned carrots after 1 yr in 1994 and 1995 were very low (0.5 ± 0.2 mg/100 g and 0.2 ± 0.03 mg/100 g), with no pattern observed in AA losses. The influence of microwave cooking on AA content in canned carrots varied considerably, with no effect observed in either year (Table 1).

**Tβ-C content.** Tβ-C concentration of F-R, FZ, and canned carrots, uncooked and microwave cooked, in 1994 and 1995 were compared (Figs. 4a, 4b, and 4c). The initial Tβ-C concentrations in freshly harvested carrots in 1994 and 1995 were 3596 ± 488 IU/100 g and 3240 ± 494 IU/100 g. There was an apparent Tβ-C increase (~10%) during the first 2 wk storage in both years. After 21 days, the concentration decreased slightly, but after 6 wk the concentration increased. Final Tβ-C concentration after 84 days in 1994 and 1995 was 4607 ± 179 IU/100 g and 4153 ± 344 IU/100 g. There was no pattern in the retention of Tβ-C in carrots during refrigerated storage. The Tβ-C content of F-R uncooked carrots was not different from microwave cooked F-R carrots (Table 2).

An increase in Tβ-C concentration was observed in steam blanched freshly harvested carrots in 1994 and 1995 ($p < 0.05$) (3617 ± 779 IU/100 g and 3922 ± 806 IU/100 g). Tβ-C concentrations of FZ steam blanched uncooked carrots after 1-d storage in 1994 and 1995 were 2294 ± 488 IU/100 g and 2932 ± 274 IU/100 g (Fig. 4b). All of the Tβ-C was retained in FZ steam blanched carrots in 1994 during 1-yr storage at –20 °C. A slight linear trend ($p < 0.05$) in Tβ-C content during the frozen storage period was found in carrots in 1994 and 1995 (Fig. 4b). Microwave cooking resulted in higher ($p < 0.05$) Tβ-C concentration in FZ carrots in both years (Table 1).

Tβ-C concentrations in carrots after hot water blanching in 1994 and 1995 were 2964 ± 283 IU/100 g and 4293 ± 136 IU/100 g; concentrations after thermal processing were 1785 ± 254 IU/100 g and 3891 ± 546 IU/100 g. The blanching procedure caused an apparent increase (4%) in Tβ-C, and thermal processing resulted in a decrease (~31%). The concentration fluctuated during the storage period (Fig. 4c). A linear decrease ($p < 0.05$) in Tβ-C concentration in canned carrots was found in 1995, but not in 1994. Microwave cooking resulted in an increase ($p < 0.05$) in Tβ-C concentration in canned carrots in 1995 (Table 2).

## Green beans

**Moisture content.** Initial moisture content (92.8 ± 0.2%) of F-R green beans was unchanged (93.6 ± 0.1%) after 16 d ($p > 0.05$) Storage of beans was discontinued after that time because they deteriorated before the projected 21-d storage elapsed. The moisture content (93.4 ± 0.3%) of FZ green beans decreased after 1 yr (91.9 ± 0.3%, $p < 0.05$). Microwave cooking decreased moisture content ~2 % in F-R and FZ green beans ($p < 0.05$)

**Total AA content.** AA concentrations of F-R and FZ green beans (uncooked and microwave cooked) for 1995 were compared (Figs. 5a and 5b). A rapid loss of AA was found during the refrigerated storage period. The initial concentration was 15.2 ± 0.6 mg/100 g; after 16 d, AA content was 1.3 ± 0.4 mg/100 g. A linear decrease ($p < 0.01$) in AA retention was found. Microwave cooking did not affect ($p > 0.05$) AA content in F-R green beans (Table 1).

Steam blanching prior to freezing slightly decreased AA content (12.6 ± 0.9 mg/100 g) in green beans. After 4-d frozen storage, AA concentration appeared to increase ($p < 0.05$ 24.7 ± 2.8 mg/100 g). However, after 1-wk storage, AA content was comparable to that immediately after blanching (11.4 ± 0.2 mg/100 g) (Fig. 5b), suggesting sample variability. AA concentration gradually decreased and the final concentration after 1 yr was 6.9 ± 0.6 mg/100 g, a linear reduction ($p<0.01$). Microwave cooking decreased ($p < 0.05$) the AA content in FZ green beans (Table 1).

**Tβ-C content.** Tβ-C concentrations of F-R and FZ green beans, uncooked and microwave cooked, were compared (Figs. 6a and 6b).

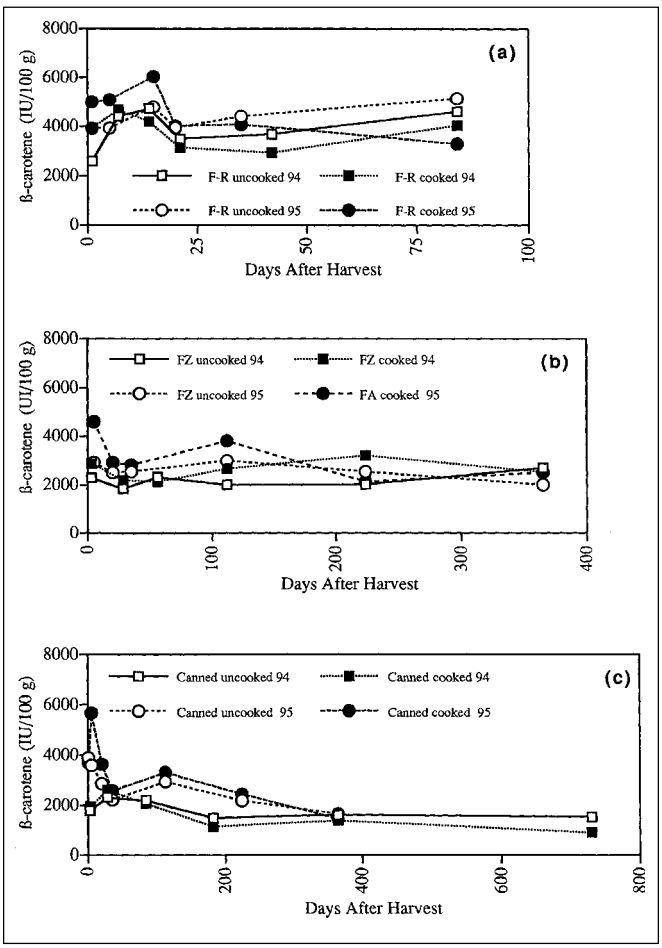


**Fig. 4—Trans β-carotene content in broccoli harvested in 1994 and 1995: (a) fresh-refrigerated (F-R) uncooked stored at 4 °C for 21 d and microwave cooked; (b) frozen (FZ) uncooked stored at –20 °C for 1 yr and microwave cooked; and (c) canned stored at 22 °C for 1 yr and microwave cooked.**

Initial Tβ-C content in F-R uncooked green beans in 1995 was 183 ± 21 IU/100 g. Final β-carotene concentration at the end of 16-d storage was 164 ± 17 IU/100 g. Mean β-carotene concentrations for the total storage time for cooked and uncooked vegetables showed that

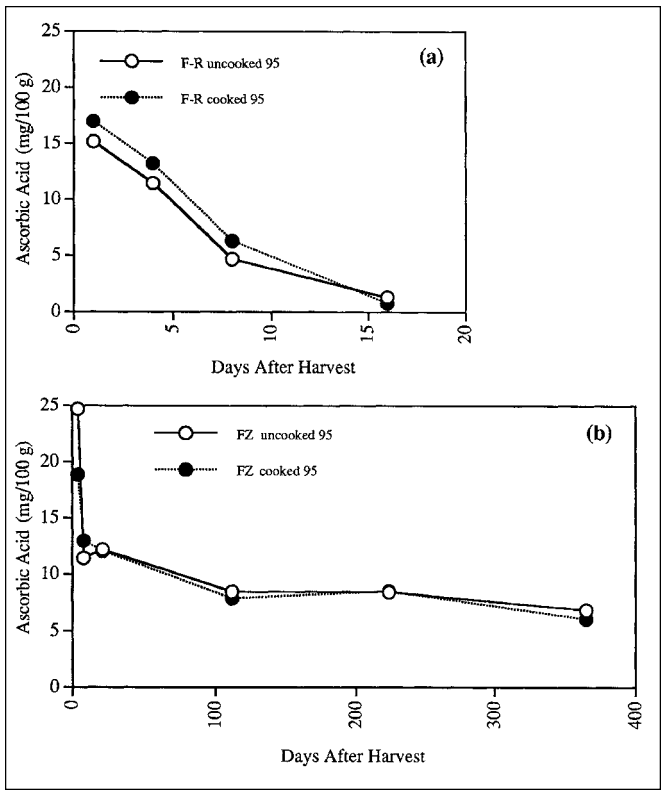


**Fig. 5—Ascorbic acid content in green beans harvested in 1995: (a) fresh-refrigerated (F-R) uncooked stored at 4 °C for 21 d and microwave cooked; (b) frozen (FZ) uncooked stored at –20 °C for 1 yr and microwave cooked.**

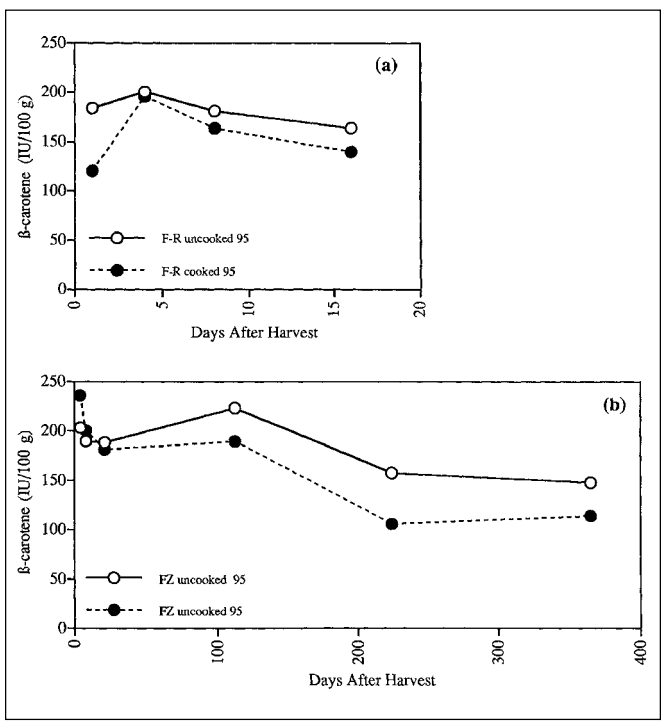


**Fig. 6—Trans β-carotene content in green beans harvested in 1995: (a) fresh-refrigerated (F-R) uncooked stored at 4 °C for 21 d and microwave cooked; (b) frozen (FZ) uncooked stored at –20 °C for 1 yr and microwave cooked.**

microwave cooking resulted in an increase ($p<0.05$) in Tβ-C concentration in F-R green beans (Table 2).

Steam blanching slightly decreased the Tβ-C content (173 ± 19 IU/100 g) of green beans. Tβ-C concentration of FZ steam blanched uncooked green beans stored at –20 °C for up to 1 yr was followed (Fig. 6b). The final Tβ-C concentration was 148 ± 14.1 IU/100 g. Green beans lost about 30% of their Tβ-C content during frozen storage. A linear decrease in the level of Tβ-C during the frozen storage period was exhibited ($p<0.01$). Microwave cooking did not affect the overall Tβ-C of FZ green beans (Table 2).

## DISCUSSION

### Moisture content

Changes in moisture content of F-R vegetables were minimized by use of microperforated polyethylene vegetable storage bags. Initial moisture in freshly harvested raw broccoli, carrots, and green beans was similar to representative values (USDA, 1984). During storage, either refrigerated or frozen, small fluctuations in moisture were most likely due to sampling variation. The range of moisture content was comparable to reported data for broccoli (Albrecht et al., 1991; Barth et al., 1992; Wu et al., 1992), carrots (Lantz, 1949), and green beans (Wu et al., 1992).

Freezing resulted in no or slight increases in moisture content of broccoli, carrots and green beans. The USDA (1984) reported slightly higher moisture content in frozen broccoli and green beans as did Wu et al. (1992). These results were postulated to be due to adsorption of water into damaged cells during steam blanching as well as water clinging to vegetable surfaces. The moisture content of C and uncooked FZ carrots was similar to that recorded by the USDA (1984). Because moisture content remained stable, variations in AA or Tβ-C concentrations during storage cannot be attributed to dehydration of tissue and increase in solids.

Microwave cooking decreased moisture content in fresh and FZ vegetables about 2% to 3%. The loss of moisture was expected because heat denatures the protein of the cytoplasm and the cell membrane. Once the cell wall is damaged, cellular fluids would exit by diffusion (Charley, 1972). Slight dehydration of vegetables during microwave heating has been commonly reported.

### Ascorbic acid

The average AA for broccoli for the 2 yr was 152 mg/100 g, considerably higher than the USDA (1984) value of 93.2 mg/100 g. It was within the range reported for 55 broccoli cultivars grown in Illinois (Kurilich et al., 1999) as well as by other researchers (Vanderslice et al., 1990). USDA (1984) values were higher for carrots (9.3 mg/100 g) and green beans (16.3 mg/100 g), but our findings for raw vegetables were in reasonable agreement with other studies (Klein and Perry, 1982; Vanderslice et al., 1990; Wu et al., 1992).

There was general confirmation of the AA content of raw broccoli, but the difference in initial AA concentration between 1994 and 1995 was notable. This could be due to natural variation, but more probably environmental conditions contributed to the effect. In 1994, broccoli with uniform heads and good yield was harvested in the fall, which is most usual in Illinois. In contrast, in March/April 1995, heavy rainfall followed by unusually high air temperatures (24 °C to 39 °C) caused severe crop stress. Heads were smaller and less uniform or compact, with fewer florets per stalk. Past research has not shown multiyear values for vitamin content, nor differences between spring and late summer plantings. It is possible that AA biosynthesis was higher in the 1995 broccoli crop, because AA is an antioxidant that protects against environmental stresses. High concentrations of AA in plant chloroplasts help protect against damaging oxygen-derived species (hydrogen peroxide, singlet oxygen, lipid peroxides, superoxide, and hydroxyl radical) that are produced in the presence of light (Halliwell, 1982).

AA is easily oxidized, so it will gradually decrease during refrigerated storage. In the vegetables we examined, AA concentration decreased during storage, but the rate, and sometimes the pattern, of decline was different for each. For example, AA concentration in F-R green beans was reduced rapidly, with more than 70% disappearing after 1-wk storage (Fig. 5a). This confirmed findings of Wu et al. (1992), who reported 58% loss AA in green beans after 3-d storage.

AA concentration in carrots decreased ~10% (1994) and ~5% (1995) during 84-d storage (Fig. 3a), but considering their low vitamin C content, such differences are unimportant. AA content in broccoli stored for 3 wk decreased ~13% (1994) and ~48% (1995) (Fig. 1a). Albrecht et al. (1991) reported that retention of AA ranged from 56% to 98% for six cultivars stored for 3 wk. The rapid loss of AA in the first 5 d of storage in 1995 may be due to the environmental stresses encountered by the broccoli before harvest. Although initial AA content was higher in 1995 than 1994, probably due to protective biosynthesis, AA might have been more easily oxidized in the fragile florets.

Although AA is considered very labile, early investigators reported minimal losses during storage (Eheart, 1969; Huguenard et al., 1955). Some reported that AA content increased in the immediate postharvest period and then gradually declined. We observed that AA content increased ~30 % in carrots (but not in broccoli or green beans) after 1 wk (1994) and 2 wk (1995) of storage. Esteve et al. (1995) showed that AA concentration in fresh green asparagus stored at 4 °C increased 2 d after harvest. Eheart and Odland (1972) reported that AA in broccoli increased about 36% between 0 and 7 days at 2 °C. Wu et al. (1992) also reported an increase in AA content of broccoli after 3 d of storage. Rate of loss of AA in vegetables measured over long-term storage (e.g., initial vs 3-wk storage or initial vs 1-yr for FZ vegetables) is thought to be first order. In most instances, changes in vitamin concentration followed a linear course. However, if we consider the fluctuations during initial days of refrigeration, the first- or second-order rates are not clear. It appears that there was variability due to the plant matrix and conditions pre- and postharvest, as well as the initial AA concentration. This makes it more difficult to develop a reliable model for AA retention during storage of refrigerated or processed vegetables.

Microwave cooking did not affect AA concentration in refrigerated vegetables. The cooked fresh vegetables had slightly higher AA content than uncooked, but differences were not always significant (Table 1). These data suggested sampling variation or AA diffusion from the cells at a different rate than other solids. The diffusion of AA out of the cells may not be as rapid as other solutes such as sugars.

Krehl and Winters (1950) stated that vegetables cooked without added water retained natural nutritive value best because losses of vitamins were held to a minimum. Kylen et al. (1961) reported that microwave cooking of fresh vegetables had no effect on AA. Erdman and Klein (1982) noted that the amount of water used in cooking, and to a lesser extent, the cooking time, affected AA losses more than the source of energy or type of cooking. If short cooking times and small amounts of water are used, more AA will be retained in any cooking method.

Steam blanching prior to freezing resulted in some loss of AA in all vegetables, due to the large surface area of the vegetables in contact with steam during blanching, as well as the thin tissue of the vegetables. Fennema (1988) suggested that steam blanching might result in less loss if it is followed by cooling that does not involve water (i.e., air cooling). In addition, cutting or slicing of intact plant tissue destroys the protective barrier provided by cellular compartmentation (Rolle and Chism, 1987). The resultant effects include leaching of nutrients, exposure to air, desiccation and interaction of enzymes, all of which can lead to loss of nutrients (Erdman and Klein, 1982).

Our results indicate that AA in processed frozen vegetables was maintained during prolonged storage. AA concentrations of carrots and green beans were not affected by freezing, and apparently increased during the first 3- to 5-d storage. AA content of FZ broccoli was higher ($p<0.05$) after 21 d (1994) and 49 d (1995). These anom-

alies could be attributed to sampling variations, which are difficult to control in nonuniform vegetables. AA concentration in all vegetables decreased slowly during 1-yr frozen storage. Our results showed that more than 50% of the initial AA content, before blanching, in broccoli was preserved in frozen processed vegetables stored 1 yr, and retention was higher in green beans. Wu et al. (1992) reported no changes in AA in broccoli and green beans after 16 wk at –20 °C. Thus, it was the blanching, not freezing, process that resulted in AA loss.

AA retention during prolonged storage was better in vegetables stored at –20 °C than during refrigerated storage at 4 °C. Frozen vegetables maintained AA content with no sign of physical and microbiological deterioration. If frozen foods are handled and processed properly, their nutritive value can be retained during storage. If enzymes that accelerate oxidation of AA are inactivated by blanching, freezing causes negligible AA losses in vegetables (Summer et al., 1983). The fact that losses of AA from vegetables are large during blanching procedures and relatively small during frozen storage suggests that losses during blanching occur primarily by leaching rather than by chemical degradation (Fennema, 1988).

### Trans β-carotene

Tβ-carotene concentrations (IU vitamin A/100 g) for each of the vegetables were lower than those reported in Handbook No. 8-11 (USDA, 1984). The differences between our values and those in the nutrient data bank were attributable to the assay method. The AOAC method gives high values for samples that contain a complex mixture of carotenoids, because the AOAC method measures total carotenes (Simpson et al., 1985). Granado et al. (1997) pointed out the HPLC methods now available are more specific than older spectrophotometric assays, making it important to revise food composition tables. The HPLC method we used was specific for Tβ-C. Values were comparable to those reported by Wu et al. (1992) and Dietz et al. (1988).

Steam blanching is thought to result in little or no loss in β-carotene content (Fennema, 1975; Gomez, 1981). β-carotene is lost as a result of heat degradation of tissue. Although β-carotene is rapidly oxidized when exposed to light and oxygen, a brief blanching treatment stabilized carotene without undue loss (Klein and Perry, 1982). Dietz and Erdman (1989) reported that steaming results in greater than 100% retention of β-carotene in vegetables, because denaturation of carotene binding proteins releases the carotenoids so that they can be extracted more easily.

Our results confirmed earlier studies of Tβ-C retention in frozen vegetables. Martin et al. (1960) reported no decrease in carotene content in broccoli stored at 0 °C for 61 wk. Wu et al. (1992) reported no difference in β-carotene content of FZ broccoli and green beans during 16-wk storage.

It is possible that thermal processing can result in losses of Tβ-C and formation of cis isomers (Borchgrevink and Charley, 1966; Chandler and Schwartz, 1988; O'Neil et al., 1991; Khachik et al., 1992; Chen and Chen, 1994). We did not attempt to quantify cis isomers, or other carotenoids in the vegetables, although small amounts were detected. Chandler and Schwartz (1988) noted that in processed sweet potatoes cis isomers could account for as much as 29% of the total β-carotene. It is also possible that some carotenoids migrated into the brine as they were released from carotene binding proteins, or that the β-carotene-protein complex was slightly water soluble. This is suggested by the fluctuations in Tβ-C content of C carrots in the days immediately after canning, until β-carotene diffusion reached equilibrium.

Broccoli showed no loss in Tβ-C content after microwave cooking confirming much earlier reports of total carotenoid retention in broccoli that had been frozen for 61 wk at –18 °C (Martin et al., 1960). Sweeney and Marsh (1971) investigated the effects of conventional cooking on provitamin A in 13 supermarket fresh, frozen, and canned vegetables. They concluded that there were no decreases in β-carotene content after cooking for 15 min. Park (1987) reported no effect on carotene content of fresh or frozen broccoli, carrots, or spinach after 6 min microwave cooking.

As with AA, we observed distinct differences in Tβ-C content between 1994 and 1995 broccoli crops. It has been suggested that under adverse cultural conditions, more β-carotene is synthesized in the cell (Krinsky, 1971). The level of β-carotene can fluctuate considerably depending on environmental conditions (Haard, 1985). However, Tβ-C increases during the first few days postharvest could result from continuation of physiochemical reactions (i.e., biochemical synthesis, metabolic interconversion, and structural rearrangement). Nutrient depletion would take place due to sustained enzyme activity, physiological degradation, and further respiration of plant tissue. β-carotene near the outer cell wall would be more susceptible to heat and oxidative damage.

Cooking has been reported to increase the chemical extractability of carotenoids, and this may be a factor in reports that cooking increases bioavailability of carotenoids in humans (Dietz et al., 1988; Hart and Scott, 1995). In our study, microwave cooking would have caused an increase in tissue degradation, which allowed greater accessibility of the β-carotene to the extracting solvent. Anderson et al. (1978), Braumann et al. (1982), and Grimme and Brown (1984) showed that carotenoids in plants are bound by protein. Heat treatments, such as cooking and steaming, help to release bound carotenoids and enables them to be more readily extracted.

Although it is possible that some interconversion of carotenoids occurs as plant cells deteriorate during storage, the apparent increase in β-carotene level that we observed may also be the result of day-to-day variation in the chromatographic system due to instability of the standard. Hart and Scott (1995) suggested that the universal availability of a suitable reference material would enhance the reliability of the data evaluation, both within and between laboratories.

### Implications

F-R broccoli, carrots, and green beans stored at 4 °C in Ziploc® vegetable bags retained moisture and Tβ-C during storage. Also, vegetables taken directly from the field to the processing plant for steam blanching and quick freezing retained over 70% of their Tβ-C during prolonged storage. Thermal processing of carrots caused the Tβ-C concentration to decrease 30%, which might be explained by conversion to the cis forms; however, the Tβ-C concentration in the canned carrots was retained during the prolonged storage period and microwave cooking. Tβ-C concentration in F-R and FZ broccoli and carrots, as well as C carrots was well preserved during microwave cooking. The Tβ-C concentration decreased in F-R and FZ green beans.

AA was most labile during processing steps that exposed large surface areas of vegetables to heat and water. Losses in blanching and cooling resulted in more losses of AA than freezing or frozen storage. Once the vegetables were frozen, AA content was relatively stable over extended storage. Refrigerated storage of broccoli for up to 3 wk resulted in only small decreases of AA, but green beans retained a very small fraction. Thus, in some cases, frozen vegetables may be better sources of AA than their fresh counterparts. A problem encountered by past researchers comparing nutrient content of fresh and processed products could be the use of vegetables of unknown variety, storage, and treatment history purchased from local supermarkets . It is not unusual for fresh produce to be in transit under variable conditions of heat, cold, and/or humidity for approximately 7 to 14 days. Under these conditions, nutrients are rapidly lost due to respiration (Wu et al., 1992). When vegetables are processed by commercial canning or freezing, postharvest handling is usually less than 12 h, so nutrient loss prior to processing is minimal.

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