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Article *in* Journal of Food Science · December 2004

DOI: 10.1111/j.1365-2621.2004.tb09919.x

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Retention of Folate, Carotenoids, and Other Quality Characteristics in Commercially Packaged Fresh Spinach

S. PANDRANGI AND L.F. LABORDE

ABSTRACT: The effect of storage temperature (4 °C, 10 °C, and 20 °C) on retention of folate, carotenoids, and other quality characteristics in commercially packaged fresh spinach were determined. Based on visual color and appearance, spinach was unacceptable after 8 d, 6 d, and 4 d at 4 °C, 10 °C, and 20 °C, respectively. Color differences (ΔE), chlorophyll degradation, fresh weight loss, and microbial populations increased at all storage temperatures and occurred more rapidly at higher temperatures. Peroxidase activity increased but was not significantly ($P > 0.05$) affected by storage temperature. Lipoxygenase activity was unaffected by storage time or temperature. Substantial losses of nutrients occurred at each storage temperature. Only 53% of folate in packaged spinach was retained after 8 d, 6 d, and 4 d at 4 °C, 10 °C, and 20 °C, respectively. Carotenoid losses increased with temperature with only 54%, 61%, and 44%, respectively, of initial detected levels remaining. Vitamin and quality changes were unaffected by presence or absence of packaging.

Keywords: carotenoids, folate, temperature, spinach

Introduction

Among fresh leafy greens, spinach is an important source of nutrients in the diet ranking 2nd behind kale in total carotenoids and folate (Holden and others 1999; USDA 2003) and 3rd in total antioxidant capacity behind only garlic and kale (Cao and others 1996). A single 30-g serving of fresh spinach containing 58 μg of folate and 2015 IU of vitamin A is equivalent to 29% and 20% of the daily value for each respective vitamin (NAS 1989).

Adequate intake of folate is an important factor in the prevention of neural tube defects such as spina bifida and anencephaly, coronary artery disease, and colorectal cancer (Herbert 1999). Carotenoids in the diet are essential for normal growth, reproduction and resistance to infection, and deficiencies have been linked to blindness and increased risk of several types of cancers (Tee 1992).

Previous studies have demonstrated that the nutrient content of fresh vegetables decreases during storage (Rodriquez-Amaya 1993; Buescher and others 1999). However, there are few studies on folate degradation in fresh produce during storage. Chen and others (1983) reported that folate in fresh spinach decreased by 26% and 27% after holding for 7 d at 4 °C or 10 h at 20 °C. Gami and Chen (1985) held Swiss chard at several temperatures and reported folate decreases of 12% after 10 d at 4 °C and 43% after 6 h at 4 °C. However, Mullin and others (1982) reported that folate levels in fresh spinach remained unchanged after storing at 4 °C for 14 d.

Ezell and Wilcox (1962) reported minimal losses of beta-carotene in kale and collard at 0 °C. However, losses increased to up to 67% after 4 d of storage at 21 °C. Little or no decreases in carotenoids have been reported in refrigerated broccoli and green beans (Wu and others 1992; Paradis and others 1996). However, Barth and Zhuang (1996) reported that total carotenoids in broccoli decreased by 42% to 57% after 6 d at 5 °C, and Howard and others (1999) reported a 64% decrease in broccoli after 21 d at 4 °C. In fresh spinach, Simonetti and others (1991) reported a 10% decrease in beta-carotene after 21 d at 4 to 6 °C. Kopas-Lane and Warthesen (1995) reported beta-carotene losses of up to 18% after 8 d at 4 °C although no changes were reported for the xanthophylls neoxanthin, violaxanthin, and lutein.

Harvested spinach leaves are transported over long distances in refrigerated trucks to the processing facility where they are sorted, washed, centrifuged to remove surface moisture, and packaged in plastic bags. Because of their high respiration rate, packages are usually ventilated to maintain aerobic conditions inside the bag.

The shelf life of spinach is less than 14 d after harvest (Kader 2002). However, quality decline may be accelerated by structural degradation, membrane lipid loss, increased respiration, and ethylene production and is sometimes accompanied by increased enzymatic activity. Strategies to increase shelf life include reducing physical damage during processing and storing at lower temperatures in modified atmospheres (Price and Floros 1993). In this study, the effects of storage temperature and time on retention of folate, carotenoids, and other quality attributes in commercially packaged fresh spinach were determined.

Materials and Methods

Sample preparation and treatments

Spinach (*Spinacia oleracea* L., var. Unipack 151) was obtained from a fresh-cut processor where it had been sorted, washed, and packed into polyethylene plastic bags (284-g capacity). Each bag contained approximately 2.5 perforations (1-mm dia) per cm^2 of package surface. After heat-sealing, the bags were immediately placed in refrigerated rooms until shipped. Bags of spinach were

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transported in insulated boxes to the Pennsylvania State Univ. Dept. of Food Science within 12 h of packaging.

Spinach in the sealed bags and spinach leaves removed from bags were placed in temperature-controlled stainless-steel chambers (Lunaire Limited, Williamsport, Pa., U.S.A.) for storage at 4 °C, 10 °C, or 20 °C. The temperature was continuously monitored using a digital temperature logger (Model HH2002AL, Omega Engineering Inc, Stamford, Conn., U.S.A.). Relative humidity inside the chamber was monitored using a Digital Humidity/Temperature Meter (Fisherbrand™, Fisher Scientific, Pittsburgh, Pa., U.S.A.). Experiments were performed in duplicate, and for each experiment, 2 random samples were removed for each assayed parameter. 20 °C samples removed for analysis at 1-d intervals and 4 °C and 10 °C samples at 2-d intervals. All absolute values are expressed on a wet weight basis to facilitate comparison to literature, whereas comparisons over time were made on dry weight basis because of significant weight loss observed during storage.

Color

Surface color of spinach leaves was measured using a spectrophotometer (Model CM 3500d Minolta Corp., Ramsey, N.J., U.S.A.) calibrated with a green standard tile (L^* = 63.6, a^* = –30.09, b^* = 8.92) as recommended by Shewfelt and others (1984). Duplicate samples of 6 to 7 randomly selected leaves were placed above the instrument so that the 30-mm aperture was completely covered. The change in total color that occurred during storage (ΔE) was calculated using the formula:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where ΔL^*, Δa^*, and Δb^* are differences in spinach color between day 0 and the sampling day (Shewfelt and others 1984).

Weight loss

At each sampling interval, spinach bags and spinach leaves were removed from the chamber and weighed using a top loading balance (Mettler PM 4600, Mettler-Toledo, Columbus, Ohio, U.S.A.). Average weight loss per sample was expressed as a percentage of initial fresh weight.

Microbial populations

Mesophilic and psychrotrophic bacterial populations were determined using the method of Garg and others (1990) with minor modifications. Spinach samples were removed from the bags, and 5 g of leaves were immediately homogenized for 1 min in 45 mL of commercial buffered peptone water (BPW, Difco, Sparks, Md., U.S.A.). Decimal dilutions were made in BPW and pour plated using plate count agar (Difco). The plates were incubated for 2 d at 30 °C for determination of mesophilic bacteria and for 14 d at 3.3 °C for psychrotrophic bacteria.

Enzyme activity

Lipoxygenase. The method of Chen and Whitaker (1986) as modified by Theerakulkait and Barrett (1995) was used. Twenty grams of spinach leaves and 2 g of polyvinylpolypyrrolidone (PVPP) were homogenized for 1 min in 40 mL of cold (4 °C) extraction buffer (0.05 *M* K_2HPO_4, 0.05 *M* citric acid, 0.86 *M* NaCl, adjusted to pH 6.4 with 2.5 *M* KOH). The homogenate was filtered through 2 layers of cheesecloth and centrifuged (4 °C) at 27000 × *g* for 30 min. The supernatant was kept on ice until analyzed.

For preparation of enzyme substrate, linoleic acid (157.2 μL) (Sigma-Aldrich Milwaukee, Wis., U.S.A.) was mixed with an equal volume of Tween-20 and 10 mL of distilled water. The mixture was clarified by adding 1.0 mL of 0.1 *N* NaOH and brought to volume with 0.2 *M* phosphate buffer (pH 7.0) in a 200-mL volumetric flask. This solution had a final concentration of 2.5 m*M* linoleic acid. The substrate solution was allowed to equilibrate to 25 °C for 10 min, flushed with O_2 for 2 min, and 0.9 mL was mixed with 0.1 mL of enzyme extract. A unit of enzyme activity is defined as that amount of enzyme that produces a change in absorbance of 0.001/min at 234 nm under the assay conditions.

Peroxidase. The previously described enzyme extract that was prepared was used for measuring peroxidase activity according to the procedure of Shue and Chen (1991) with minor modifications. The substrate was prepared by mixing 558 μL of guaiacol (Sigma-Aldrich) with 194.4 μL of 30% H_2O_2 (Sigma-Aldrich) and brought to volume with 0.2 *M* sodium phosphate buffer (pH 6.0) in a 100-mL volumetric flask to obtain a final concentration of 0.05 *M* guaiacol and 0.2 *M* H_2O_2. Three milliliters of substrate was mixed with 25 μL of enzyme extract and the reaction at 25 °C was monitored for 3 min at 420 nm. A unit of enzyme activity is defined as the amount of enzyme that produces a change in absorbance of 1.0/min at 420 nm under assay conditions.

Gas composition

O_2 and CO_2 concentrations in packages of spinach were measured using Mocon PAC CHECK 450 and 550, respectively, gas analyzers (Modern Controls Inc., Minneapolis, Minn., U.S.A.). Gas samples (5 or 8 cc, respectively) were withdrawn from a single perforation on each package using the automatic sampler mode. Measurements were made at 3 different perforations on each package and an average value was determined. O_2 and CO_2 concentrations in the storage chambers were also measured.

Folate analysis

Total folate in spinach samples was determined by enzymatic digestion of the tissue matrix to release bound folate vitamers followed by microbiological assay using the 96-well microplate procedure described by Tamura (1990) and modified by Pandrangi and LaBorde (2004). The microbiological method for vitamin quantification uses the growth response of folate-dependent *Lactobacillus rhamnosus* in food sample extracts that have been enzymatically treated to release the bound vitamin. Spinach leaves (10 ± 0.01 g) were homogenized in a blender with 50 mL of 0.1 *M* phosphate buffer containing 114 m*M* ascorbic acid (Sigma-Aldrich) (final pH 4.1). The homogenate was heated in a water bath at 100 °C for 10 min and immediately cooled for storage at –70 °C. Folate was enzymatically released from the tissue matrix by combining 250 μL of the homogenate with an equal volume of 0.3 *M* citric acid buffer (pH 4.0) and 500 μL of protease (20 mg/mL) and then incubating at 37 °C for 8 h. At the end of incubation period, protease was denatured by heating the sample at 100 °C for 5 min in a water bath. After cooling to room temperature (approximately 23 °C), 200 μL of protease-treated sample was mixed with 950 μL of 0.3 *M* phosphate buffer (pH 7.0) and 50 μL of conjugase (from rat serum, Harlan Bioproducts, Indianapolis, Ind., U.S.A.) and then incubated at 37 °C for 3 h. Total folate in each enzyme-treated sample was determined by microbiological assay using *L. rhamnosus* and 5-formyl tetrahydrofolate (5-HCO-H_4PteGlu, calcium salt) as the folic acid standard. Turbidimetric growth after 18 h at 37 °C was compared by measuring absorbance at 490 nm using a 96-well microplate reader (Model 315, Bio-tek Instruments, Hercules, Calif., U.S.A.). Pooled human blood plasma was used as an internal standard and assay validity was confirmed by determining the folate content of triplicate samples of infant formula obtained from the Natl. Inst. of Standards and Technology (standard reference material nr 1846, NIST, Gaithersburg, Md., U.S.A.).

Carotenoid and chlorophyll analysis

Sample extraction and high-performance liquid chromatography (HPLC) analysis were achieved using the method of Bushway (1986) modified by Kopas-Lane and Warthesen (1995). Spinach extracts were prepared by adding 60 mL of cold (4 °C) methanol/tetrahydrofuran (1:1 vol/vol) containing 20 g of sodium sulfate and 1 g of magnesium carbonate to 10 g of spinach leaves and homogenizing for 1 min in a Brinkmann Polytron (Model PT 10/35, Brinkmann Instruments Inc., Westbury, N.Y., U.S.A.) at a speed setting of 6. The homogenate was filtered through a Whatman nr 42 filter and the residue was re-extracted twice. The filtrate was transferred to a 200-mL volumetric flask and diluted to volume with the homogenizing solvent. A 5-mL aliquot was dried under nitrogen, and the residue was dissolved in 1 mL of methanol. The solubilized pigments were filtered through 0.45-mm Gelman membrane filter before HPLC injection.

The reverse-phase HPLC system consisted of Waters 510 series pumps connected to a Waters 717 autosampler (Waters Inc, Milford, Conn., U.S.A.). The gradient solvent system consisted of 90% acetonitrile/water/methanol (90/5/5, vol/vol) at a flow rate of 1.5 mL/min reaching 100% methanol in 15 min. Pigments were separated on a C-18 column (Model 218TP54, Grace Vydac Inc, Hesperia, Calif., U.S.A.), and spectra were obtained using a Waters 996 photodiode array detector for peak identification at 436 nm. Spectra and retention times of chlorophyll a and b and trans beta-carotene were compared with standards (Sigma Aldrich, Milwaukee, Wis., U.S.A.). All other identifications were based on published spectral information (Braumann and Grimme 1981; Quackenbush 1987). Quantities of total carotenoids and xanthophylls in treated spinach samples were compared by calculating changes in peak areas for each of the identified compounds.

Statistical analysis

The data were analyzed within individual temperatures using Analysis of Covariance. Differences between packaged and unpackaged samples were analyzed using Dunnett's test (Minitab, Minitab Inc., State College, Pa., U.S.A.). Differences at the maximum storage time at each temperature were compared using 1-way analysis of variance (ANOVA).

Results and Discussion

Spinach characteristics

Leaves were considered unacceptable for commercial sale when they became noticeably wilted and curled along the edges with approximately 5% of the leaves showing signs of yellowing. Shelf life parameters used in this study are similar to those used by Piagentini and others (2002) to describe loss of quality in fresh-cut spinach during refrigerated storage. Based on preliminary visual observations, spinach was considered commercially unacceptable after 8 d, 6 d, and 4 d at 4 °C, 10 °C, and 20 °C, respectively. These respective storage times were designated as shelf life values for each temperature and were used as maximum storage times in subsequent experiments.

Changes in visual quality were confirmed by objective measurements. Color differences (ΔE) increased at all storage temperatures and were most rapid at higher temperatures (Figure 1). However, after 8 d, 6 d, and 4 d at 4 °C, 10 °C, and 20 °C, respectively, ΔE values did not significantly ($P > 0.05$) differ from each other. Gnanasekharan and others (1992) similarly reported that ΔE values for fresh spinach increased more rapidly under temperature abuse conditions compared with refrigeration.

The total amount of chlorophyll initially contained in spinach was 332.8 ± 17 and 65.5 ± 5 μg/g, respectively. The ratio of chlorophyll a to b remained approximately 5:1 throughout the study. Chlorophyll decreased ($P \leq 0.05$) with increasing storage time and degradation was more rapid at higher temperatures (Figure 2). Only 75%, 69%, and 58% of the initial amount remained after storing for 8 d, 6 d, or 4 d at 4 °C, 10 °C, or 20 °C. Results from chlorophyll determination and color change (Figure 2) indicate that chlorophyll levels at the end of each spinach shelf life at 4 °C, 10 °C, and 20 °C do not correlate with ΔE values.

Weight loss, caused by evaporative loss of moisture, significantly ($P \leq 0.05$) increased with storage time and temperature (Figure 3). After 8 d, 6 d, and 4 d at 4 °C, 10 °C, and 20 °C, fresh weights did not differ significantly ($P > 0.05$) from each other. Loss of water

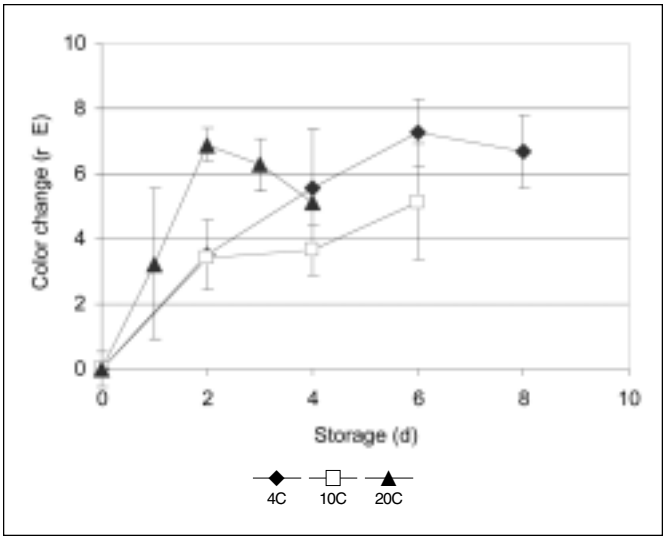

Figure 1 — Change in total color (ΔE) of packaged spinach stored at 4, 10, and 20 °C for up to 8, 6, and 4 d, respectively. Each value represents the mean of 4 determinations at each temperature ± SE.

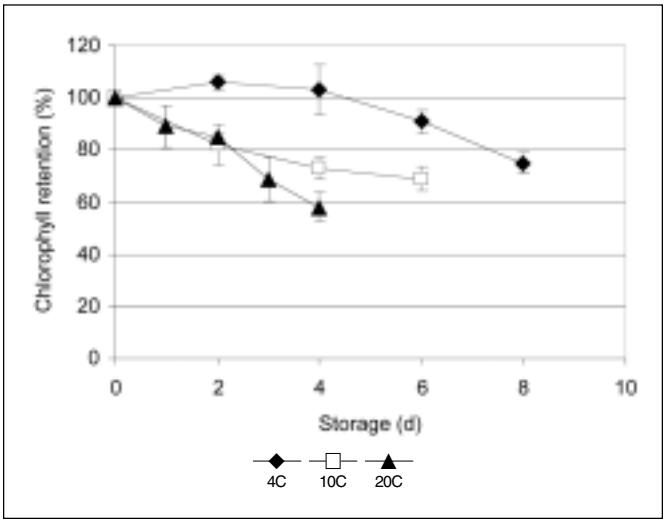

Figure 2 — Retention of chlorophyll (%) in packaged spinach stored at 4, 10, and 20 °C for up to 8, 6, and 4 d, respectively. Each value represents the mean of 4 determinations each temperature ± SE. Initial total chlorophyll content was 399 ± 18 μg/g.

through transpiration can be attributed to differences in internal relative humidity of the tissue with that of the storage chamber, which remained within a narrow relative humidity range of 55% to 58% at all temperatures. The approximately equal loss of moisture at the end of the shelf life for each temperature is consistent with the equally wilted appearance of the leaves at the same storage times and temperatures.

Microbial populations

Mesophilic and psychrotrophic bacteria populations in stored spinach are shown in Table 1. The amount of mesophilic bacteria populations initially contained in packaged spinach varied between 5.0 and 6.0 logs. Garg and others (1990) reported a similar range for microbial populations in salad vegetables in the packinghouse. Microbial populations in spinach stored at 4 °C, 10 °C, and 20 °C increased by 1.6, 1.1, and 2.3 logs, respectively, with final levels reaching 7.1 to 7.5 logs.

Psychrotrophic populations similarly increased from 3.6 to 5.9 logs initially to a maximum of 6.9 to 7.5 logs. Other studies have reported similar increases in mesophilic and psychrotrophic bacteria on spinach leaves under refrigerated (5 °C to 7 °C) and temperature abuse (10 °C) conditions (Garg and others 1990; Babic and others 1996; Piagentini and others 2003).

Enzyme activity

Lipoxygenase activity did not significantly ($P > 0.05$) change during storage at each of the temperatures studied (data not shown). However, activity tended to be higher in spinach leaves stored at 20 °C compared with 10 °C or 4 °C. In contrast, peroxidase activity increased with storage time for spinach leaves stored at 4 °C and 10 °C (Figure 4). Increases in peroxidase activity in stored spinach have been reported (Baardseth and von Elbe 1989) although both enzymes may participate in degradation reactions (Rodriquez-Amaya 1993).

Gas composition of packages

Concentrations of oxygen and carbon dioxide inside packages were not significantly ($P > 0.05$) affected by storage time or temperature (data not shown). Mean O_2 and CO_2 levels inside the packages were 20.1% and 0.03%, respectively, and did not significantly ($P > 0.05$) differ from air inside the chamber that surrounded the packages. Fresh vegetables respire during storage and, in sealed packages, can result in depletion of oxygen and accumulation of carbon dioxide (Price and Floros 1993). These results suggest that the perforated packaging film provided little resistance to diffusion of gases between the respiring tissue and the atmosphere surrounding the bags.

Folate and carotenoid loss

Total folate in packaged spinach samples taken over the entire experiment ranged between 84 and 225 μg/100 g with a mean value of 160 ± 42 μg/100 g. Previous studies have reported folate values for fresh spinach from 161 to 410 μg/100 g (Klein an others 1981; Mullin and others 1982; Aiso and Tamura 1998; Lin and Lin 1999; Shrestha and others 2000; Iwatani and others 2003; USDA 2003; Pandrangi and LaBorde 2004). Lower folate concentrations in fresh spinach before storage in this study may be the result of intrinsic differences between spinach cultivars, growing and handling conditions, or by vitamin degradation during minimal processing and storage (Mullin and others 1982).

Folate levels decreased ($P \leq 0.05$) with increasing storage time at approximately the same rate for each temperature (Figure 5). After 8 d, 6 d, or 4 d at 4 °C, 10 °C, or 20 °C, folate remaining at each temperature was an average of 53% of the initial amount. Chen and others (1983) reported that fresh spinach held in a refrigerator (4 °C) for 7 d showed a 26%, reduction in folate.

All-trans beta-carotene, 9-cis beta-carotene, and the xanthophylls neoxanthin, violaxanthin, and lutein were detected in spinach samples (Figure 6). The same carotenoids were reported by Kopas-Lane and Warthesen (1995). Values for beta-carotene in fresh spinach before storage ranged from 54 to 127 μg/g with a mean value of 89.7 ± 23 μg/g. These values are comparable to other reported ranges of 30 to 82 μg/g (Quakenbush 1987; Masrizal and others 1997; Holden and others 1999).

Total carotenoids in spinach samples, compared at each time by adding individual HPLC peak areas, decreased ($P \leq 0.05$) as stor-

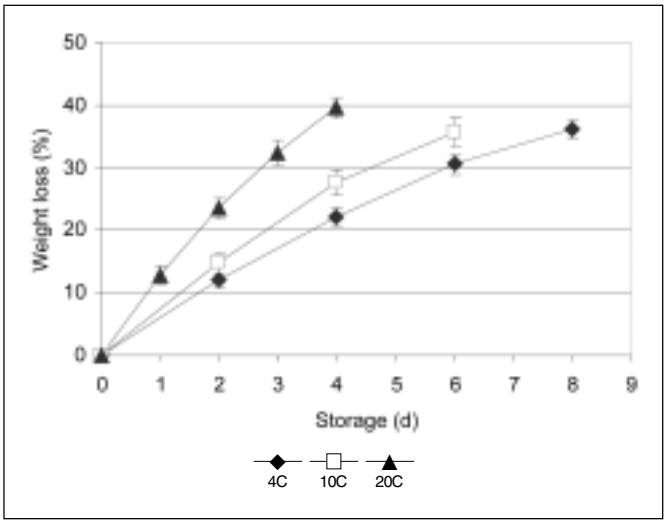

Figure 3 — Fresh weight loss in packaged spinach stored at 4, 10, and 20 °C for up to 8, 6, and 4 d, respectively. Each value represents the mean of 10 determinations at each temperature ± SE.

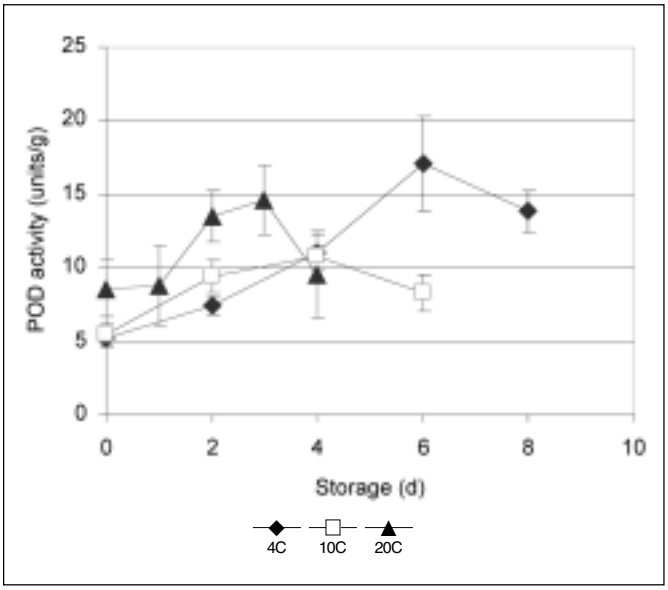

Figure 4 — Peroxidase (POD) activity of packaged spinach stored at 4, 10, and 20 °C for up to 8, 6, and 4 d, respectively. Each value represents the mean of 4 determinations at each temperature ± SE.

Table 1 — Growth of mesophilic and psychrotrophic bacteria in packaged spinach stored at 4, 10, and 20 °C for up to 8, 6, and 4 d, respectively.

4 °C			10 °C			20 °C		
Storage Time (d)	Mesophiles	Psychrotrophs	Storage Time (d)	Mesophiles	Psychrotrophs	Storage Time (d)	Mesophiles	Psychrotrophs
	Log_{10} CFU/g			Log_{10} CFU/g			Log_{10} CFU/g	
0	5.9 A[1]	5.5 A	0	6.0 A	5.9 A	0	5.00 A	3.6 A
2	6.4 A	5.5 A	2	7.0 B	7.0 B	1	7.3 B	7.2 B
4	5.7 A	5.3 A	4	7.2 B	7.2 B	2	7.4 B	7.4 B
6	7.5 B	7.4 B	6	7.1 B	7.0 B	3	7.5 B	7.4 B
8	7.5 B	7.5 B	—	—	—	4	7.3 B	6.9 B

[1] Values in a column followed by different letters are significantly different ($p \leq 0.05$)

age time increased and degraded more rapidly at higher temperatures (Figure 7). After 8 d, 6 d, and 4 d of storage at 4 °C, 10 °C, and 20 °C, respectively, total carotenoids retained were 54%, 61%, and 44% of initial detected levels. All-trans beta-carotene levels ranged from 84% to 34% of initial levels after storage between 4 °C and 20 °C, respectively (Table 2) and this isomer was more stable than the 9 –cis form. Retention of xanthophylls, determined by comparing HPLC peak areas, was also enhanced at lower storage temperatures. Mean retention values for the 3 compounds were 44% after 4 d at 20 °C, 59% after 6 d at 10 °C, and 65% after 8 d at 4 °C. Kopas-Lane and Warthesen (1995) reported comparable losses of beta-carotene in spinach stored at 4 °C. However, they did not observe significant losses of neoxanthin, violaxanthin, or lutein.

Lower losses of folate (Mullin and others 1982; Chen and others 1983) and carotenoids (Simonetti and others 1991; Kopas-Lane and Warthesen 1995) have been reported by others using similarly stored fresh spinach. In these studies, store-bought or field-grown spinach was used that was sorted immediately before experiments began to remove damaged or discolored leaves. In the present study, the entire content of packages of spinach was used and may have included leaves that had already lost moisture to evaporation or had been damaged during washing, centrifuging, and packaging. The greater loss of nutrients in this study is consistent with reports of more rapid quality decline in wilted or wounded vegetables compared to tissues that are not wilted or damaged (Ezell and Wilcox 1962; Yang 1985; Price and Floros 1993).

Packaging effect

There were no significant ($P > 0.05$) differences for all parameters studied between packaged and unpackaged spinach (data not

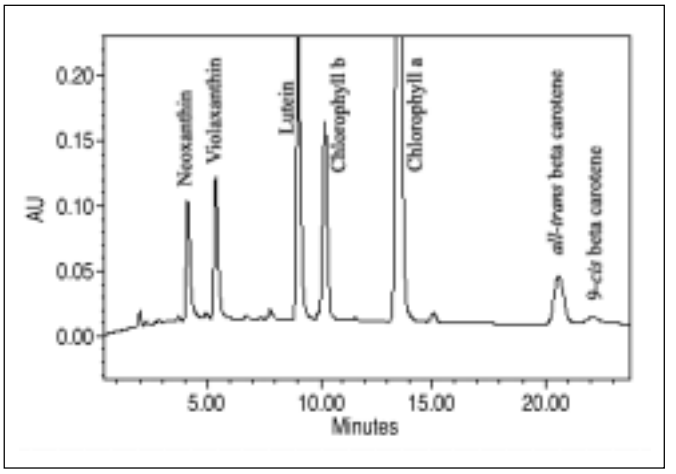

Figure 6 — Chromatogram of spinach pigments.

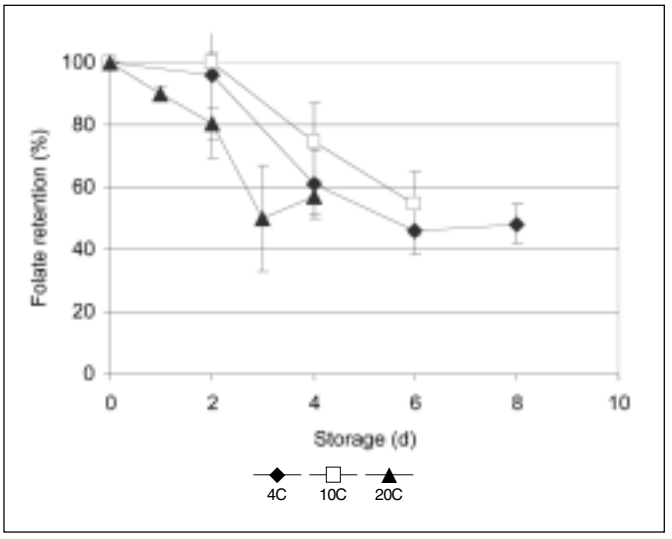

Figure 5 — Retention of total folate in packaged spinach stored at 4, 10, and 20 °C for up to 8, 6, and 4 d, respectively. Each value represents the mean of 4 determinations at each temperature ± SE.

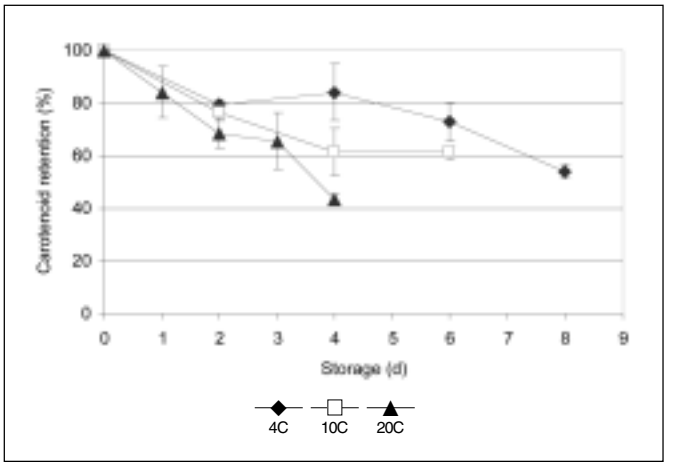

Figure 7 — Retention of total carotenoids in packaged spinach stored at 4, 10, and 20 °C for up to 8, 6, and 4 d, respectively. Each value represents the mean of 4 determinations at each temperature ± SE.

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Table 2 — Retention of carotenoids in packaged spinach stored at 4, 10, or 20 °C for 8, 6, or 4 d, respectively.

Storage conditions	Carotenoids remaining (%)				
	All-trans beta-carotene	9-cis beta-carotene	Neoxanthin	Violaxanthin	Lutein
4 °C / 8 d	84.3 A	49.9 A	65.1 A	69.1 A	61.0 A
10 °C / 6 d	40.9 B	52.4 AB	57.5 A	55.4 AB	65.0 A
20 °C / 4 d	34.3 B	33.4 B	41.5 B	41.3 B	48.3 B

[1] Values in a column followed by different letters are significantly different ($p \leq 0.05$)

shown). Diffusion of moisture and gasses through the bag perforations is apparently unimpeded as evidenced by identical fresh weight losses and gas compositions in packaged and unpackaged samples. Gas composition in minimally processed vegetables is known to strongly influence the retention of vitamins and other quality characteristics during storage (McGill and others 1966; Barth and others 1993; Barth and Zhuang 1996; Howard and Hernandez-Brenes 1998). The absence of differences in microbial populations, chlorophyll loss, color, enzyme activity, and vitamin retention between packaged and nonpackaged spinach in this study is consistent with the identical fresh weight losses and gas compositions observed.

Conclusions

Minimally processed vegetables are attractive to consumers because of their convenience and nutritional value. However, substantial losses of folate and carotenoids occurred in packaged spinach under both refrigerated and temperature abuse conditions. Published values for these nutrients in fresh spinach may, therefore, not always accurately reflect levels that are consumed. It is, therefore, essential that growers, packers, fresh-cut processors, and retailers maintain storage temperatures as low as possible to minimize vitamin losses in fresh spinach. Consumers should keep fresh spinach refrigerated and use the product as close as possible to the time at which it was purchased.

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