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Ascorbic Acid, Carotenoids, and Visual Quality of Baby Spinach as Affected by Shade Netting and Postharvest Storage

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Baby spinach (*Spinacia oleracea* L.) was grown under three types of shade netting (high transmittance, spectrum-altering, and low transmittance) to study the effect on the concentrations of vitamin C (ascorbic acid and dehydroascorbic acid), carotenoids, and chlorophyll and on the visual quality of the leaves. The spinach was sown in April and August and harvested at two growth stages. After harvest, leaves were stored in polypropylene bags at 2 and 10 °C. Shading significantly decreased the ascorbic acid concentration of April-sown spinach by 12–33%, but in the August-sown spinach, the response was inconsistent. Concentrations of total carotenoids and total chlorophylls were significantly higher under the nettings in many cases, especially under the spectrum-altering and low-transmittance nettings. Postharvest visual quality and postharvest persistence of the compounds analyzed were not greatly affected by shading. We conclude that these shade nettings are acceptable to use in baby spinach production when it comes to the studied aspects of internal and external quality of the produce.

KEYWORDS: ***Spinacia oleracea* L.; bioactive compounds; vitamin C; light quality**

INTRODUCTION

A high intake of fruit and vegetables has been correlated with a low incidence of a number of chronic diseases, including cardiovascular disease and some forms of cancer (*1–5*). This protective effect has been attributed to a wide range of bioactive compounds found in fruit and vegetables, including those with antioxidant action, such as ascorbic acid (vitamin C) and carotenoids (*6*, *7*). β-carotene is the most extensively studied carotenoid with regard to the prevention of cardiovascular disease and cancers. Epidemiological studies mainly show positive health effects from carotenoids (*8*, *9*), but some supplementation trials have shown adverse effects (*10*, *11*). For ascorbic acid, there have not been only positive effects reported either; a high intake has been correlated to a decreased risk of cardiovascular disease, cancer, and cataracts (*12*), but there are also contradictory results (*13*). The overall findings indicate that it may be better to eat fruit and vegetables than to rely on supplements for the intake of these compounds (*14*).

Spinach (*Spinacia oleracea* L.) is a leafy vegetable that is mainly supplied fresh or frozen to the consumer. Baby spinach is harvested after a short growth period, and the leaves are thus relatively small. This product is marketed fresh in polypropylene bags and has become increasingly popular in the past few years (*15*). Spinach is considered to be of high nutritional value, containing high concentrations of carotenoids and ascorbic acid, as well as folate and minerals (*16*).

Shade netting is used in southern Europe mainly to protect cultures of lamb's lettuce [*Valerianella locusta* (L.) Lat.] against light stress and mechanical damage from severe weather conditions. This practice is still not very common in northern Europe, although an increased use is expected for a range of horticultural crops. Shade netting not only decreases light quantity but also alters light quality to a varying extent and might also change other environmental conditions. However, little is known about how various types of netting affect the biochemical quality of crops and the persistence of quality postharvest. Protection of the leaves against mechanical damage may prevent an increase in respiration during storage (*17*), thereby maintaining a high postharvest quality (*18*). Furthermore, protection against rain splashing may decrease the risk of produce being contaminated with potential pathogens present in the soil (*19*).

The concentration of bioactive compounds in fruit and vegetables is known to vary with factors such as genotype, climate, cultivation practices, growth stage at harvest and postharvest handling, and storage (*20–23*). We have previously studied changes in some quality parameters in baby spinach as a function of the plant growth stage and time of season (*24*). The highest levels of ascorbic acid were found in the youngest plants, whereas the concentrations of most carotenoids were higher in the older plants. Furthermore, postharvest storage may

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Table 1. Temperature and Light Conditions during Field Cultivation of Baby Spinach at Two Sowing Times[a]

	August 2003	April 2004
mean temperature (°C)	16.7	11.3
temperature range (°C)	5.3–28.6	3.1–24.3
mean photoperiod[b] (h:min)	15:38	17:21
photoperiod range[b] (h:min)	14:31–16:46	16:01–18:37
mean global radiation (kW m^{-2} day $^{-1}$)	4.2	5.1
total radiation (kW m^{-2})	121	178

[a] Values are from sowing until the second harvest. [b] Civil twilight to civil twilight.

cause changes in the concentrations of ascorbic acid and carotenoids (*24–26*).

Light intensity can affect the concentrations of carotenoids and ascorbic acid in vegetables. High light intensities, absorbed in excess of that used by the plant for photosynthesis, may cause photo-oxidative damage. The excess radiation generates reactive oxygen species that may cause photoinhibition if not scavenged, for example, by carotenoids (*27*). The xanthophyll cycle may also dissipate the excess energy (*28*). Leaves grown in the sun generally exhibit a larger pool of the xanthophyll cycle pigments than leaves grown in the shade (*29*, *30*). Ascorbic acid is also involved in protection against damage from excess light, both as an antioxidant and as a cofactor for violaxanthin de-epoxidase, which is involved in the xanthophyll cycle (*31*, *32*). Light intensity is generally positively related to the ascorbic acid concentration of fruit and vegetables (*33*).

The aim of the present study was to investigate how shading affects the concentration of carotenoids and ascorbic acid in baby spinach and the persistence of visual and biochemical quality postharvest. Our research hypothesis is that netting will alter crop light conditions and microclimates and will be expected to influence the concentrations of ascorbic acid and carotenoids, as well as spinach postharvest visual quality.

MATERIALS AND METHODS

Field Experimental Setup. Spinach (*Spinacia oleracea* L.) cv. Emilia was cultivated on a field bed in southern Sweden (55°39′ N 13°08′ E). The spinach was sown on August 12th, 2003, and harvested after 21 and 28 days, or sown on April 21st, 2004, and harvested after 27 and 34 days. Seeds were sown on a soil bed, 20 cm high, 100 cm wide at the top, and 120 cm wide at the base. The sowing density was 650 seeds/m^2, and the soil was a loamy sand with 3–5% organic matter. Prior to sowing, the soil was analyzed for available nutrients, and mineral fertilizers were added accordingly up to 100 kg/ha (64 kg N/ha supplied as KNO_3 in August and 88 kg N/ha as NPK 11–5–18 in April). Temperature and light conditions during the growing periods are given in **Table 1**.

The spinach was covered with three types of shade netting or left uncovered (control). The shade netting was placed over the beds within one week of sowing, and prior to emergence. The netting was draped on plastic arches inserted at a spacing of about 100 cm along the bed and anchored firmly to the ground using tent pegs. The netting tunnel was approximately 50 cm high. The field experiment was a randomized block design with four replicates per treatment (growth stage × shading), repeated at two sowing dates. After harvest, the material was pooled to a representative sample within each treatment and harvest before further analyses, and postharvest treatments were performed.

Shade Netting. Three types of shade netting (PE 30.15 B7 high transmittance, F.1132 spectrum-altering, FOM 40 low transmittance) were obtained from the technical textiles manufacturer Diatex, St. Genis Laval, France. The figure names are from the manufacturer, and the characterization names of the nettings are only used in this investigation. The nettings not only decrease light quantity but also alter spectrum quality. Characteristics of the three types of netting are given in **Table 2**, and the microclimate under the nettings and for the unshaded control are given in **Table 3**. The effects of the nettings on spectrum quality are shown in **Figure 1**.

Table 2. Characteristics of the Three Types of Shade Netting Used

netting type	color	transmittance[a]	weight[b]	product name
high transmittance	black/green	74%	70 g m^{-2}	FOM40
spectrum-altering	green	65%	100 g m^{-2}	F1132
low transmittance	black/olive green	61%	85 g m^{-2}	PE30. 15B7

[a] Average in the 400–700 nm range on a sunny day. [b] According to manufacturer.

Harvest. After visual assessment of the leaf size, the first harvest (stage I) was performed 21 or 27 days after sowing in August and April, respectively. The second harvest (stage II) was 7 days later. Leaves were harvested manually using sharp knives and were transferred to dark, cool rooms at a temperature of 2 °C within one hour of harvest.

Postharvest Treatment. After cooling, a portion of the leaves was frozen at –80 °C for chemical analyses. For the studies on changes during storage, fresh leaves were placed in plastic containers (6 × 13 × 22 cm) covered in oriented polypropylene (OPP) film (Amcor, Melbourne, Australia) using an industrial machine. Each container contained 100 g of leaves. The containers were stored in a dark climate chamber (Model SWTA02, Svalöf Weibull AB, Svalöv, Sweden) at 2 or 10 °C for 6 or 9 days, simulating practical commercial conditions. The weight loss during storage was determined by weighing the filled containers before and after storage. The dry matter content was determined by drying at 70 °C to a constant weight.

Vitamin C Extraction and Analysis. Vitamin C, ascorbic acid, and dehydroascorbic acid were determined in fresh and stored samples. Samples of August-sown spinach were prepared in triplicate and April-sown in quadruplicate. All steps in the analysis were performed under dim green light. A total of 4 g of freshly chopped leaves were placed into a plastic bottle together with 20 mL of *meta*-phosphoric acid (1.5%) and were homogenized with an Ultra Turrax apparatus (Model TP1810, IKA Werke GmbH, Staufen, Germany) for 1 min. Samples were stored in the freezer at –80 °C prior to high-performance liquid chromatography (HPLC) analysis. Before HPLC analysis, the samples were thawed in lukewarm water and centrifuged for 8 min at 12 900*g*, after which, 5 mL of each supernatant was filtered through a Sep-pak Plus C_{18} cartridge (Waters, Milford, MA), previously conditioned with 10 mL of methanol and 5 mL of water. The first 4 mL of each sample were discarded, and the remaining 1 mL was collected in a microcentrifuge tube. An aliquot of the collected extract was used for HPLC analysis, as described below. The dehydroascorbic acid concentration was calculated by subtracting the ascorbic acid concentration from the total vitamin C concentration, obtained after a reduction procedure (*34*). For this procedure, an aliquot of the extract was added to a 1% dithiothreitol solution and allowed to react for 30 min at pH 7.0, where the pH was adjusted with K_2HPO_4, prior to HPLC analysis.

Samples were analyzed on an Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) HPLC, using an isocratic method with a flow rate of 1.2 mL/min. The mobile phase consisted of 25% $NH_4H_2PO_4$ (15 mM) and 75% acetonitrile, with the pH adjusted to 3.9. A 20 μL portion was injected from each sample. A Waters carbohydrate analysis column, 300 × 3.9 mm with a particle size of 10 μm, was used. Absorbance was measured at 248 nm. Samples were quantified using an external ascorbic acid standard (Merck, Darmstadt, Germany).

Carotenoid Extraction and Analysis. Prior to extraction, an aliquot of the frozen leaf material was freeze-dried and ground into a fine powder. The samples were extracted and analyzed under dim green light, with three replicates for August-sown spinach and four replicates for April-sown. A total of 40 mg of the freeze-dried sample was extracted three times, consecutively with 500 μL of 90% ethanol, 10% hexane, and 0.1% butylated hydroxytoluene. Samples were extracted in an ultrasonicator (Branson 2200, Branson, Danbury, CT) for 40 +

Table 3. Effects of the Three Types of Netting on Microclimate at the Four Harvest Occasions

	sowing time	height[a]	unshaded	high transmittance	spectrum-altering	low transmittance
mean temperature[b] (°C)	August 2003	25 cm	16.1	16.0	16.3	16.0
		5 cm	16.5	17.1	17.2	16.5
		−10 cm	17.1	16.9	17.3	16.9
	April 2004	25 cm	12.8	12.8	13.0	12.9
		5 cm	13.8	13.7	13.4	13.6
		−10 cm	15.0	14.0	14.0	13.5
relative humidity[b] (%)	August 2003	25 cm	84.7	83.1	79.9	81.8
		5 cm	82.2	82.0	83.1	83.5
	April 2004	25 cm	79.4	76.0	77.6	77.2
		5 cm	74.6	74.0	73.9	75.4

[a] Above or below ground level. [b] Values are from sowing until the second harvest.

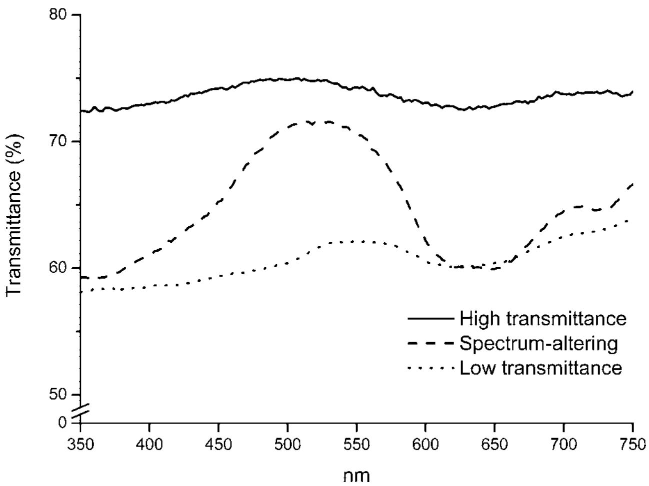

Figure 1. Transmittance through the netting types on a sunny day.

30 + 20 min. After each extraction, samples were centrifuged at 12 900*g* for 5 min. The supernatants from the three consecutive extractions were pooled.

Carotenoids were analyzed by reversed-phase HPLC, using a method described by Kidmose et al. (*35*) with some minor modifications. Mobile phase eluents were A, 80% methanol/20% water, and B, 100% ethyl acetate. The flow rate was 1.2 mL/min with an injection volume of 20 μL. The mobile phase gradient was 20% eluent B for 4 min, a change to 70% eluent B over the next 20 min, 70% eluent B for 2 min, a change to 20% eluent B over the next 2 min, and 20% eluent B for 6 min. The column was a Phenomenex Nucleosil 5 C18 (250 × 4.6 mm, 5 μm, Phenomenex, Torrance, CA), with a security guard column, Phenomenex C18 ODS, 4 × 3.0 mm. The carotenoid concentration was quantified using an external β-carotene standard (Sigma, St. Louis, MO). The absorbance was measured at 445 nm using a diode array detector (Waters, Milford, MA). Carotenoids were identified by a comparison of chromatographic retention times and spectral characteristics with those reported in the literature (*35*).

Chlorophyll Extraction and Analysis. Chlorophyll was analyzed on the day after harvest in both August- and April-sown plants, and after 6 and 9 days of storage in August-sown plants only, using the method described by Hendry and Price (*36*). The extraction solution consisted of 81.8% acetone, 18.0% water, and 0.2% ammonium hydroxide. Analyses were performed in triplicate under dim green light. A handful of leaves was chopped finely, and 250 mg were homogenized in a cooled mortar with 3 mL of an extraction solution. The extract was transferred into centrifuge tubes, and the remaining chlorophyll in the mortar was rinsed into the centrifuge tubes with the extraction solution. Samples were centrifuged at 4 °C and 12 900*g* for 8 min. The supernatant was transferred to a volumetric flask, and the volume was increased up to 10 mL with the extraction solution. The absorbance was measured at 480, 645, 663, and 710 nm, using a Varian Cary 50 spectrophotometer. The chlorophyll concentration was calculated according to the formula given by MacKinney (*37*).

Visual Quality. The visual quality of the harvested product was evaluated in fresh material and after 6 and 9 days of storage. Overall visual quality of the plant material was scored on a 1–9 scale, where 9 = excellent, 7 = good (some leaves are slightly yellowed or decayed or there is a slight loss of turgor), 5 = fair (the limit of marketability), 3 = poor (most leaves are yellowed or decayed and there is a considerable loss of turgor), and 1 = extremely poor (unusable).

Statistical Analysis. Data were statistically evaluated using an analysis of variance. A statistical analysis of the data showed significant interactions between shade treatments, sowing times, growth stages, and postharvest storage conditions. The least significant difference (LSD) was used to compare the mean values of the treatment combinations. All statistical analyses were performed using the statistical software MINITAB 14 (Minitab Inc., State College, PA). A significance level of 5% was used unless otherwise indicated. For the LSD comparisons, a Bonferroni correction was applied for mass comparisons.

RESULTS

Vitamin C. In the April-sown spinach, the ascorbic acid and total vitamin C concentrations per unit of dry weight of fresh leaves were significantly higher in unshaded plants than in those under the shade nettings (**Table 4**). This difference was especially evident on the second harvesting occasion (stage II). At the first harvest (stage I), shading decreased the ascorbic acid concentration of April-sown spinach by 12–15% and, at stage II, by 21–33%. In August-sown spinach, the vitamin C concentration was significantly higher in the unshaded plants than in the shaded, only at growth stage II. At stage I, the difference between the treatments was very small. Shading gave between a 17% increase and a 27% decrease in ascorbic acid concentration in the August-sown crop (**Table 4**). In the August-sown spinach, the dehydroascorbic acid/vitamin C ratio was higher in the unshaded plants and in those grown under the high-transmittance netting than in those grown under the low-transmittance netting, and especially under the spectrum-altering netting (**Table 4**). Differences were smaller in the April-sown crop (**Table 4**).

In general, the concentrations of ascorbic acid and total vitamin C were higher in the younger plants (stage I) than in the older (stage II). In August-sown spinach, the concentrations decreased considerably in both the unshaded and shaded treatments from stage I to stage II. In the April-sown crop, the changes were smaller but statistically significant in the three shaded treatments.

The vitamin C and ascorbic acid concentrations per unit of dry weight generally decreased during the postharvest storage of both shaded and unshaded leaves, especially at 10 °C (**Figure 2**). However, ascorbic acid and vitamin C concentrations did not always decrease throughout the storage period in this study. The concentrations generally decreased from harvest until 6 days after the harvest, but in some cases, they increased from 6 to 9 days after the harvest (**Figure 2**). This increase during the end

Table 4. Vitamin C, Ascorbic Acid, and Dehydroascorbic Acid (DHA) Concentration (mg/g dry weight) in Baby Spinach cv. Emilia Sown on Two Dates, Cultivated under Three Different Types of Shade Netting or Unshaded, and Harvested at Two Growth Stages (I, II)[a]

sowing	stage	netting	vitamin C		ascorbic acid		DHA		DHA/vitamin C	
August 2003	I	unshaded	7.86 ± 0.01	a	6.25 ± 0.32	ab	1.62 ± 0.31	a	0.21 ± 0.04	a
		high transmittance	7.91 ± 0.08	a	6.21 ± 0.26	a	1.70 ± 0.21	a	0.21 ± 0.03	a
		spectrum-altering	7.91 ± 0.09	a	7.29 ± 0.07	c	0.62 ± 0.14	b	0.08 ± 0.02	b
		low transmittance	7.72 ± 0.07	a	6.68 ± 0.13	b	1.04 ± 0.20	c	0.13 ± 0.02	c
August 2003	II	unshaded	5.93 ± 0.02	a	4.50 ± 0.17	a	1.43 ± 0.17	a	0.24 ± 0.03	a
		high transmittance	4.76 ± 0.08	b	3.29 ± 0.03	b	1.47 ± 0.08	a	0.31 ± 0.01	a
		spectrum-altering	5.59 ± 0.09	c	4.72 ± 0.14	a	0.87 ± 0.15	b	0.16 ± 0.03	b
		low transmittance	5.38 ± 0.03	c	3.75 ± 0.17	a	1.07 ± 0.14	a	0.20 ± 0.03	a
April 2004	I	unshaded	8.85 ± 0.22	a	8.33 ± 0.14	a	0.53 ± 0.10	a	0.06 ± 0.01	a
		high transmittance	7.98 ± 0.13	b	7.36 ± 0.08	b	0.62 ± 0.07	a	0.08 ± 0.008	a
		spectrum-altering	7.66 ± 0.32	b	7.06 ± 0.29	b	0.60 ± 0.04	a	0.08 ± 0.002	a
		low transmittance	7.80 ± 0.13	b	7.22 ± 0.14	b	0.56 ± 0.02	a	0.07 ± 0.004	a
April 2004	II	unshaded	8.98 ± 0.23	a	8.27 ± 0.19	a	0.71 ± 0.11	a	0.08 ± 0.01	a
		high transmittance	7.24 ± 0.12	b	6.53 ± 0.09	b	0.70 ± 0.07	a	0.10 ± 0.008	a
		spectrum-altering	6.06 ± 0.09	c	5.52 ± 0.11	c	0.55 ± 0.05	a	0.09 ± 0.008	a
		low transmittance	6.64 ± 0.16	c	5.85 ± 0.12	c	0.79 ± 0.06	a	0.12 ± 0.006	a

[a] Values are mean ± standard deviation. Values within each growth stage, sowing time, and column followed by the same letter are not significantly different ($p \leq 0.05$).

of the storage period only occurred at the lower temperatures (2 °C). No consistent differences in changes during storage between the unshaded and the shaded plants were found in the present study. The concentrations of vitamin C and ascorbic acid were considerably more stable during the storage of April-sown spinach than that of the August-sown (**Figure 2**).

Carotenoids. The major carotenoid found in the baby spinach leaves was lutein (all-*trans*-lutein + 13-*cis*-lutein, on average 39% of the total carotenoids at harvest, measured as β-carotene equivalents), followed by violaxanthin (all-*trans*-violaxanthin, 28% of total), β-carotene (all-*trans*-β-carotene + 9-*cis*-β-carotene, 18%), and neoxanthin (9′-*cis*-neoxanthin, 12%, **Table 5**). Antheraxanthin (all-*trans*-antheraxanthin) and lutein 5,6-epoxide (13-*cis*-lutein 5,6-epoxide) were detected in small amounts.

Total carotenoid concentration was similar or significantly higher in the shaded leaves than in the unshaded, depending on the sowing time, growth stage, and type of netting (**Table 5**). The spectrum-altering and low-transmittance netting gave the highest total carotenoid concentrations, although not always significantly different from the other treatments (**Table 5**). The individual carotenoids showed similar responses to shading as the total carotenoid concentration.

The violaxanthin concentration was higher in August-sown spinach than in the April-sown (**Table 5**). The concentrations of the other carotenoids and the total carotenoid concentration, on the other hand, were most often higher in April-sown spinach than in the August-sown.

The concentration of each of the carotenoids and the total carotenoid concentration decreased from stage I to stage II in April-sown spinach, while in August-sown spinach, the individual carotenoids showed varying patterns (**Table 5**). Violaxanthin and the total carotenoid concentration decreased significantly from stage I to stage II, whereas lutein did not change significantly and neoxanthin and β-carotene increased.

The total carotenoid concentration and the concentrations of the individual carotenoids were often higher after 6 days of storage than in the fresh material (**Figure 3**). The total carotenoid concentration then often decreased again to about the same level as in the fresh material or a lower level after 9 days of storage. In some cases, however, the concentrations continued to increase from 6 to 9 days of storage. The individual carotenoid compounds showed similar changes during storage (data not shown).

Visual Quality. The fresh leaves scored between 9 and 7 on the nine-grade scale. The visual quality generally decreased during storage to a higher extent in August-sown spinach than in the April-sown (data not shown). After 6 days of storage of both April- and August-sown spinach, leaf quality had decreased by zero or one points on the nine-grade scale for all of the netting types (data not shown). Generally, there was no difference in quality score between the two storage temperatures. After 9 days of storage, the visual quality had decreased by up to three points in the August-sown crop, and by up to two points in the April-sown, compared to the original score. Score 5 was considered the limit of saleability. For stage I August-sown spinach after 9 days of storage at 10 °C, the score was 5 or lower, irrespective of shade treatment. At stage II, the score was also 5 after 9 days at 2 °C. For the April-sown crop, only stage I under the low-transmittance netting scored as low as 5.

Storage temperature affected the visual quality in different ways for August and April sowing. In the August-sown crop, the leaves stored at 10 °C scored lower than the leaves stored at 2 °C for all the netting types at stage I. At the second harvest, there was no difference, except in the unshaded plants, where the higher storage temperature gave a lower quality score. In April-sown spinach, however, the leaves stored at 10 °C surprisingly scored one point higher on the quality scale than those stored at 2 °C, except at stage II for the spectrum-altering and low-transmittance netting. There were no consistent differences in quality persistence during storage between the leaves grown under the different netting types or between the shaded and the unshaded leaves.

DISCUSSION

Vitamin C. Light conditions have previously been found to affect the ascorbic acid concentration of fruit and vegetables, with decreasing light intensity generally giving a decreased ascorbic acid concentration (*33*). Increasing the photon flux density by 400% has been shown to increase the ascorbic acid concentration of spinach by about 50% (*38*). The higher ascorbic acid concentration at higher light intensities may be associated with the role of ascorbic acid in preventing damage from excess light (*31*, *32*). However, shading might have different effects at different light intensities. In this study, irradiation was higher in April than in August (**Table 1**), and it is possible that the more consistent response to the shade netting in April-sown spinach than in August-sown was due to the higher irradiation.

The greater dehydroascorbic acid/vitamin C ratio in the unshaded leaves and those grown under the high-transmittance

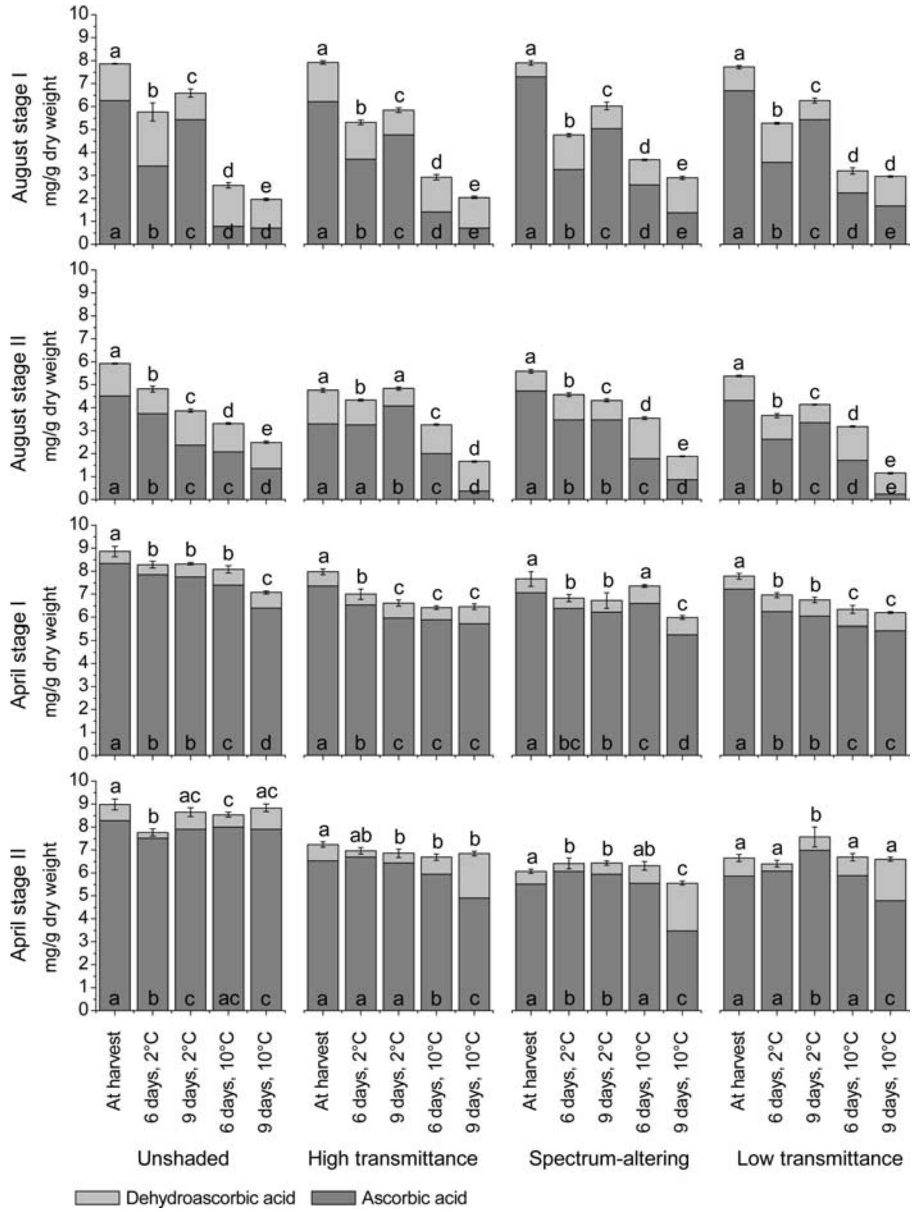

Figure 2. Vitamin C concentration (ascorbic acid and dehydroascorbic acid) in fresh and stored baby spinach leaves sown and harvested at different times and cultivated under three different types of shade netting. Bars designated by the same letter within each sowing time, harvest, and shade treatment combination are not significantly different. Letters inside bars represent ascorbic acid, and those on top of the bars represent vitamin C. Error bars represent standard deviation.

netting (**Table 4**) may have implications for human nutrition. Although the vitamin activity is essentially the same for ascorbic acid and dehydroascorbic acid (*39*), the antioxidant capacity is much higher for ascorbic acid. However, recent investigations indicate that the uptake of dehydroascorbate in humans is different from the uptake of ascorbate (*40*) and that dehydroascorbate might affect human pentose phosphate pathway and glutathione levels (*41*). Therefore, the human physiological effects of changes in the dehydroascorbic acid/vitamin C ratio seem to be complex.

Previous studies on spinach have generally found decreasing concentrations of vitamin C and ascorbic acid during postharvest storage (*20*, *24*, *25*, *42–44*). In this study, however, ascorbic acid and vitamin C concentrations did not always decrease throughout the storage period (**Figure 2**). The higher ratio of dehydroascorbic acid to vitamin C in the spinach stored at 10 °C than that at 2 °C indicates a higher rate of degradation of ascorbic acid, and it is possible that an increase in vitamin C concentration might have occurred also at 10 °C, but before day 6, and was therefore not recorded. Metabolic processes are generally slower at lower temperatures (*45*). Since the dry matter content in the OPP bags changed very little during storage, these results may indicate that there was a net synthesis of ascorbic acid during storage. An increased ascorbic acid concentration after harvest has previously been observed in light-exposed barley (*46*). However, the spinach leaves in the present study were stored in the dark. Toledo and co-workers (*47*) reported a decreasing ascorbic acid concentration in young spinach leaves during storage at 8 °C in the dark as well as in the light.

We have previously reported that the concentration of ascorbic acid at harvest is positively correlated with visual quality after storage, possibly due to protection from ascorbic acid against oxidative stress during storage (*24*, *42*). In the present study, the initial concentration of ascorbic acid was generally higher in April than in August, and the dehydroascorbic acid/vitamin C ratio lower, except under the spectrum-altering netting at the Stage I harvest. Visual quality was also better maintained in the April-sown spinach than in the August-

Table 5. Carotenoid Concentration (mg/g dry weight) in Baby Spinach cv. Emilia Sown on Two Dates, Cultivated under Different Types of Shade Netting or Unshaded, and Harvested at Two Growth Stages (I, II)[a]

sowing and stage	netting	total		lutein[b]		violaxanthin[c]		β-carotene[d]		neoxanthin[e]	
August 2003 Stage I	unshaded	1.19 ± 0.05	a	0.41 ± 0.02	a	0.42 ± 0.02	a	0.19 ± 0.008	a	0.13 ± 0.007	a
	high transmittance	1.24 ± 0.05	ab	0.44 ± 0.02	ab	0.42 ± 0.02	a	0.20 ± 0.009	b	0.14 ± 0.005	b
	spectrum-altering	1.25 ± 0.02	ab	0.44 ± 0.006	b	0.42 ± 0.006	a	0.20 ± 0.004	b	0.14 ± 0.002	b
	low transmittance	1.27 ± 0.04	b	0.45 ± 0.02	b	0.43 ± 0.01	a	0.20 ± 0.007	b	0.15 ± 0.006	b
August 2003 Stage II	unshaded	1.13 ± 0.02	a	0.41 ± 0.006	a	0.33 ± 0.006	a	0.21 ± 0.002	a	0.14 ± 0.003	a
	high transmittance	1.17 ± 0.03	ab	0.43 ± 0.01	ab	0.34 ± 0.008	a	0.22 ± 0.004	ab	0.15 ± 0.004	b
	spectrum-altering	1.20 ± 0.03	b	0.44 ± 0.01	b	0.35 ± 0.01	a	0.22 ± 0.005	b	0.15 ± 0.005	b
	low transmittance	1.25 ± 0.06	c	0.47 ± 0.02	c	0.36 ± 0.02	b	0.22 ± 0.01	b	0.16 ± 0.008	c
April 2004 Stage I	unshaded	1.26 ± 0.04	a	0.52 ± 0.02	a	0.30 ± 0.009	a	0.24 ± 0.005	a	0.16 ± 0.005	a
	high transmittance	1.37 ± 0.002	b	0.58 ± 0.001	b	0.32 ± 0.001	ab	0.25 ± 0.001	a	0.17 ± 0.001	b
	spectrum-altering	1.45 ± 0.04	b	0.61 ± 0.02	b	0.34 ± 0.01	c	0.27 ± 0.008	b	0.18 ± 0.004	b
	low transmittance	1.40 ± 0.02	b	0.60 ± 0.01	b	0.33 ± 0.005	bc	0.25 ± 0.003	a	0.17 ± 0.002	b
April 2004 Stage II	unshaded	1.10 ± 0.03	ab	0.46 ± 0.01	a	0.26 ± 0.008	ab	0.20 ± 0.006	a	0.14 ± 0.004	ab
	high transmittance	1.07 ± 0.02	b	0.44 ± 0.004	a	0.25 ± 0.004	a	0.20 ± 0.002	a	0.13 ± 0.008	a
	spectrum-altering	1.20 ± 0.05	c	0.50 ± 0.02	b	0.29 ± 0.01	c	0.21 ± 0.009	a	0.16 ± 0.006	c
	low transmittance	1.15 ± 0.02	ac	0.48 ± 0.01	ab	0.28 ± 0.005	bc	0.21 ± 0.004	a	0.15 ± 0003	bc

[a] Values are mean ± standard deviation. Values within each growth stage, sowing time, and column followed by the same letter are not significantly different ($p \leq 0.05$). [b] All-*trans*-lutein + 13-*cis*-lutein. [c] All-*trans*-violaxanthin. [d] All-*trans*-β-carotene + 9-*cis*-β-carotene. [e] 9′-*cis*-neoxanthin.

sown. A high concentration of ascorbic acid at harvest may indicate that the concentration is more likely to be retained during storage, although this may not be the only explanation. There may be a difference in activity of enzyme systems that reduce dehydroascorbic acid to ascorbic acid, such as the ascorbate-glutathione cycle (*48*).

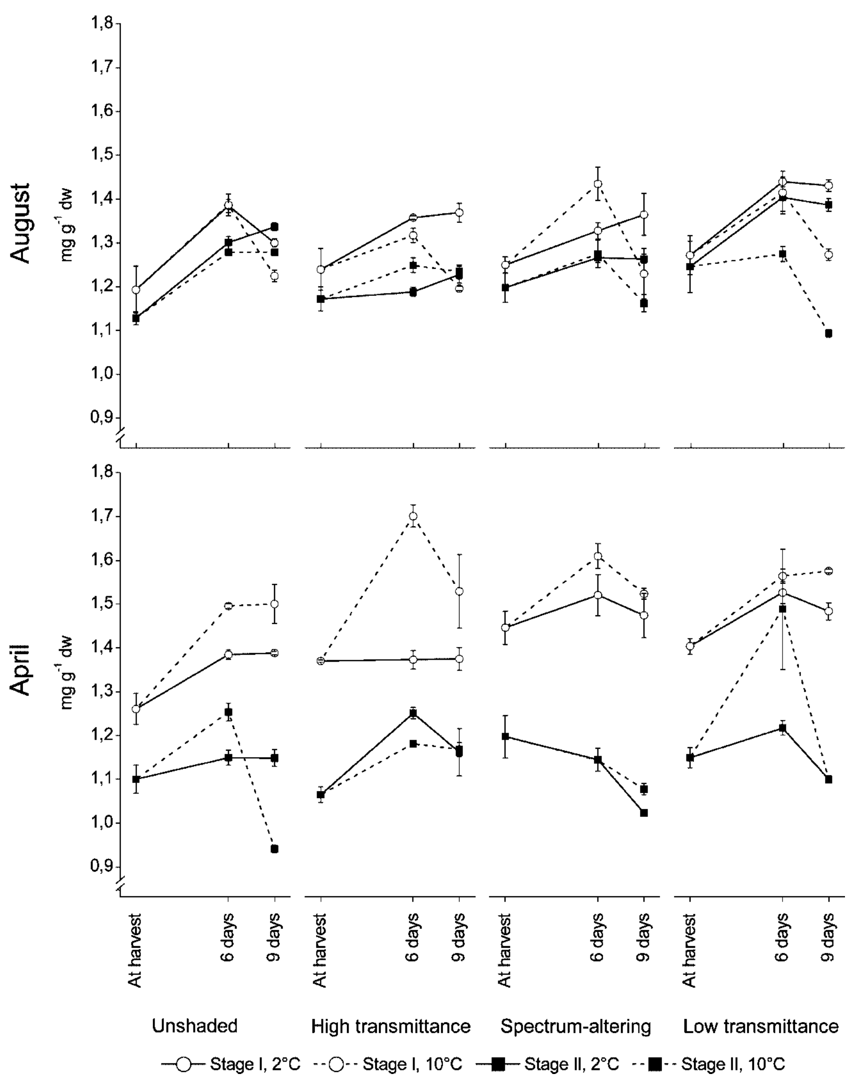

Figure 3. Carotenoid concentration in fresh and stored baby spinach leaves sown and harvested at different times and cultivated under three different types of shade netting. Error bars represent standard deviation.

Table 6. Total Chlorophyll Concentration (mg/g dry weight) and Dry Matter (%) of Baby Spinach cv. Emilia Sown on Two Dates, Cultivated under Different Types of Shade Netting, and Harvested at Two Growth Stages (I, II)[a]

sowing	stage	netting	Total chlorophyll		dry matter
August 2003	I	unshaded	7.92 ± 0.09	a	8.1
		high transmittance	8.44 ± 0.24	ab	7.2
		spectrum-altering	8.34 ± 0.35	a	7.1
		low transmittance	9.02 ± 0.28	b	7.1
August 2003	II	unshaded	8.89 ± 0.27	a	7.3
		high transmittance	9.21 ± 0.47	a	6.6
		spectrum-altering	10.14 ± 0.45	b	6.5
		low transmittance	10.62 ± 0.32	b	7.0
April 2004	I	unshaded	10.94 ± 0.28	a	9.5
		high transmittance	11.83 ± 0.48	ab	8.7
		spectrum-altering	12.96 ± 0.74	bc	8.4
		low transmittance	13.24 ± 0.76	c	8.4
April 2004	II	unshaded	8.91 ± 0.43	a	12.1
		high transmittance	10.86 ± 0.68	b	9.8
		spectrum-altering	11.10 ± 0.13	b	8.6
		low transmittance	11.28 ± 0.57	b	9.2

[a] Values are mean ± standard deviation. Values within each growth stage and sowing time followed by the same letter are not significantly different ($p \leq 0.05$).

Carotenoids. The higher carotenoid concentration in the shaded leaves than that in the unshaded appears to contradict reports of higher levels of carotenoids in plants grown in the sun than in the shade (*29*, *30*). However, those studies report the concentration per unit of leaf area. As the dry matter content is generally higher in sun leaves, the carotenoid concentrations per dry weight are generally higher in shade leaves than in sun leaves. In photosynthesis, carotenoids are involved in light harvesting and photoprotection (*49*). The xanthophylls violaxanthin, antheraxanthin, and zeaxanthin have a special role in the xanthophyll cycle, protecting against excess excitation energy and reactive oxygen species formed during light harvesting in photosynthesis. In addition, the xanthophyll lutein has been suggested to contribute to photoprotection by the de-epoxidation of lutein 5,6-epoxide into lutein (*50*). In accordance with previous results reported (*35*), lutein 5,6-epoxide was detected in spinach in this investigation, in the range 0.016–0.027 mg/g of dry weight. However, no consistent difference in concentration between unshaded leaves and those grown under the different nettings could be detected (data not shown) that could indicate a photoprotective role of this compound in baby spinach.

The fact that the violaxanthin concentration was higher in the August-sown spinach than in the April-sown is somewhat surprising, as the need for xanthophyll cycle carotenoids would be larger at higher light intensities, compared to other carotenoids (*49*). Violaxanthin was therefore not expected to occur at higher concentrations in the August-sown spinach than in the April-sown.

The higher concentrations of total and individual carotenoids after 6 days of storage than those in the fresh material suggest a postharvest synthesis of carotenoids. Other studies on leafy vegetables have mainly reported decreased or unchanged carotenoid concentration during storage (*26*, *51*, *52*). Other bioactive compounds, such as flavonoids, have been reported to sometimes increase during the storage of leafy vegetables (*53*, *54*). Since this investigation concerns baby spinach, the leaves studied are relatively young compared to those studied in other investigations, a fact that may have a bearing on the changes in concentrations of carotenoids and other bioactive compounds during storage.

Concluding Remarks. Shade netting may be used to protect cultures from severe weather conditions, prevent mechanical damage, or improve hygiene. In this study, shading was found to have different effects on the parameters studied. The ascorbic acid concentration was slightly lower under the shade netting compared to that of unshaded plants, whereas the carotenoid concentration was higher. Yield (values not shown), postharvest visual quality, and postharvest persistence of the compounds analyzed were not greatly affected by shading. Other factors, such as sowing time and postharvest storage temperature, apparently have a greater influence. For the parameters studied in this paper, we conclude that the use of these types of shade netting in baby spinach cultivation is acceptable.

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