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# Carotenoid Composition of Hydroponic Leafy Vegetables

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Because hydroponic production of vegetables is becoming more common, the carotenoid composition of hydroponic leafy vegetables commercialized in Campinas, Brazil, was determined. All samples were collected and analyzed in winter. Lactucaxanthin was quantified for the first time and was found to have concentrations similar to that of neoxanthin in the four types of lettuce analyzed. Lutein predominated in cress, chicory, and roquette (75.4 ± 10.2, 57.0 ± 10.3, and 52.2 ± 12.6 $\mu$g/g, respectively). In the lactucaxanthin-containing lettuces, $\beta$-carotene and lutein were the principal carotenoids (ranging from 9.9 ± 1.5 to 24.6 ± 3.1 $\mu$g/g and from 10.2 ± 1.0 to 22.9 ± 2.6 $\mu$g/g, respectively). Comparison of hydroponic and field-produced curly lettuce, taken from neighboring farms, showed that the hydroponic lettuce had significantly lower lutein, $\beta$-carotene, violaxanthin, and neoxanthin contents than the conventionally produced lettuce. Because the hydroponic farm had a polyethylene covering, less exposure to sunlight and lower temperatures may have decreased carotenogenesis.

**KEYWORDS: Carotenoids; leafy vegetables; hydroponic**



## INTRODUCTION

Leafy vegetables are important year-round sources of vitamins, minerals, fiber, and other phytochemicals with health-promoting effects, such as carotenoids and polyphenols. $\beta$-Carotene, the most potent provitamin A, and lutein, the carotenoid implicated in the reduced risk of cataract and macular degeneration together with zeaxanthin (*1*), are the principal carotenoids of leaves.

The carotenoid composition varies markedly as influenced by such factors as variety, part of the plant utilized, degree of maturity at harvest, climatic or geographic effects, and cultivation and postharvest handling practices (*2*). Data on the influence of these factors are necessary in efforts to enhance the carotenoid levels of foods.

Leafy vegetables are increasingly produced by hydroponic farming. This new method of production has several advantages: (a) smaller area required and greater productivity per area; (b) possibility of using areas not suitable for traditional farming; (c) possibility of several harvests during the year because of rapid plant growth; (d) crop rotation not necessary; (e) less consumption of water and fertilizer; (f) greater hygiene and less possibility of contamination with microorganisms, nematodes, and insects inherent to the soil (consequently, the use of fungicides, bactericides, and insecticides, as well as herbicides, is totally eliminated or reduced); (g) less manpower needed; and (h) greater control of quality. Some disadvantages are the (a) high cost of installation, (b) dependence on electricity in automated systems, and (c) need for specialized laborers. In Brazil, the system is not automated and, according to the producers, the cost of production is similar to that of conventional farming.

Few papers on the composition of hydroponic vegetables were found in the literature. Sweet potato greens from hydroponic (nutrient film technique) and bed plants were analyzed for dry matter, protein, ash, total dietary fiber, fat, minerals (Ca, Fe, K, Na, Mg, and Zn), vitamins (total carotene, ascorbic acid, and thiamin), oxalic and tannic acids, and chymotrypsin and trypsin inhibitors (*3*). Differences in the nutrient and antinutrient concentrations were observed in the three cultivars studied due to variety and production method. It was concluded that the nutritional quality of the hydroponic greens was better than that of the bed greens. Hydroponic water dropwort leaves were found to have high contents of ascorbic acid and ash (*4*). The median nitrate-N concentration found in hydroponic lettuce was more than twice the median concentration of field-grown lettuce, but the number of samples of hydroponic produce analyzed was small (5). The $\beta$-carotene content of hydroponic butter head lettuce ranged from 18 to 28 $\mu$g/g and that of soil-grown lettuce varied from 8 to 31.9 $\mu$g/g (*6*). Hydroponic and nonhydroponic tomatoes appeared to have the same lycopene content (*7*), averaging 36.15 ± 4.17 and 36.25 ± 1.24 $\mu$g/g, respectively.

It has also been shown that hydroponic conditions can be modified so as to increase the concentrations of desirable constituents and lessen undesirable components. For example, hydroponic spinach with high vitamin C and low $NO_3$ contents can be produced by transferring the plants to N-free medium prior to harvest (*8*).

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The present study was carried out to determine the carotenoid composition of marketed hydroponic leafy vegetables and to compare the carotenoid levels of hydroponic and field-grown lettuce.

## MATERIALS AND METHODS

**Sampling and Sample Preparation.** To determine the carotenoid composition of marketed vegetables, the samples were purchased from a supermarket at different times during the winter season (average minimum and maximum temperatures of 13 and 24 °C), always in the morning, a few hours after harvest. Analysis was carried out on arrival at the laboratory.

For each vegetable, five sample lots (collected at different times) were analyzed individually. Each lot (consisting of two bunches) was finely cut and mixed, and 2−5 g samples were taken for analysis.

For the comparison of hydroponic and conventionally produced lettuce, three samplings were carried out. At each sampling time, three sample lots of curly loose-head lettuce were taken from a hydroponic farm and three sample lots of the same lettuce variety of equivalent maturity were taken from a neighboring conventional farm, which was not irrigated. The lots were analyzed individually. Each lot consisted of five heads of lettuce collected from different parts of the farm. The five heads were cut and mixed, and 5 g samples were taken for analysis.

**Analysis.** The carotenoid composition was determined according to a procedure described previously (*9*). This involved isolation of standards by open column chromatography and quantitative analysis by high-performance liquid chromatography (HPLC). The carotenoids were extracted with cold acetone, partitioned to petroleum ether, concentrated in a rotary evaporator, and dried under $N_2$. The residue was redissolved in 2 mL of HPLC grade acetone; 1 mL was filtered through a 0.22 $\mu$m PTFE syringe filter (Millipore), and then 10 $\mu$L was immediately injected into the liquid chromatograph automatically.

The carotenoids were identified as described in detail by Rodriguez-Amaya (*10*) with the combined use of chromatographic data (HPLC retention times and thin-layer chromatography $R_f$ values on silica gel plates developed with 5% methanol in toluene), cochromatography with authentic carotenoids, the visible absorption spectra obtained spectrophotometrically and by the photodiode array detector, and, for the xanthophylls, chemical tests, for example, acetylation with acetic anhydride of secondary hydroxyl groups, methylation with acidic methanol of allylic secondary hydroxy groups, and epoxide−furanoid rearrangement of 5,6-epoxy groups with dilute HCl. The carotenoids were isolated by open column chromatography on an MgO:Hyflosupercel column to obtain the visible spectra in petroleum ether (PE) and to carry out the chemical reactions.

Zeaxanthin ($\beta,\beta$-carotene-3,3′-diol) and *cis*-isomers of $\beta$-carotene were also detected but were not quantified because they were present at very low levels. The $\beta$-carotene concentration reported in this paper, therefore, refers to *trans*-$\beta$-carotene.

The concentrations of the carotenoid standards were determined by visible absorption spectrometry, using the following $A^{1\%}_{1cm}$ values: $\beta$-carotene, 2592 in PE; lutein, 2550 in ethanol; violaxanthin, 2550 in ethanol; neoxanthin, 2243 in ethanol. For lactucaxanthin a $A^{1\%}_{1cm}$ value of 2944 in PE was calculated according to the formula that relates the absorption coefficient and the molecular masses of two carotenoids of the same chromophore (*11*), using the $A^{1\%}_{1cm}$ of 3120 of $\epsilon,\epsilon$-carotene.

**HPLC Conditions.** The HPLC analysis was performed on a Waters separation module (model 2690) equipped with an autosampler injector, controlled by a Millenium 2010 workstation, using a monomeric $C_{18}$ column (Spherisorb S3 ODS2), 3 $\mu$m, 4.6 × 150 mm. The mobile phase consisted of acetonitrile, methanol, and ethyl acetate, containing 0.05% of triethylamine (TEA), used at a flow rate of 0.5 mL/min. A concave gradient (curve 10) was applied from 95:5:0 to 60:20:20 in 20 min, maintaining this proportion until the end of the run. Reequilibration took 15 min. A UV−visible photodiode array detector (Waters model 996) was used. Detection was at the wavelengths of maximum absorption of the carotenoids in the mobile phase (maximum plot): neoxanthin, 438 nm; violaxanthin, 441 nm; lactucaxanthin, 439 nm; lutein, 447 nm; and $\beta$-carotene, 454 nm.

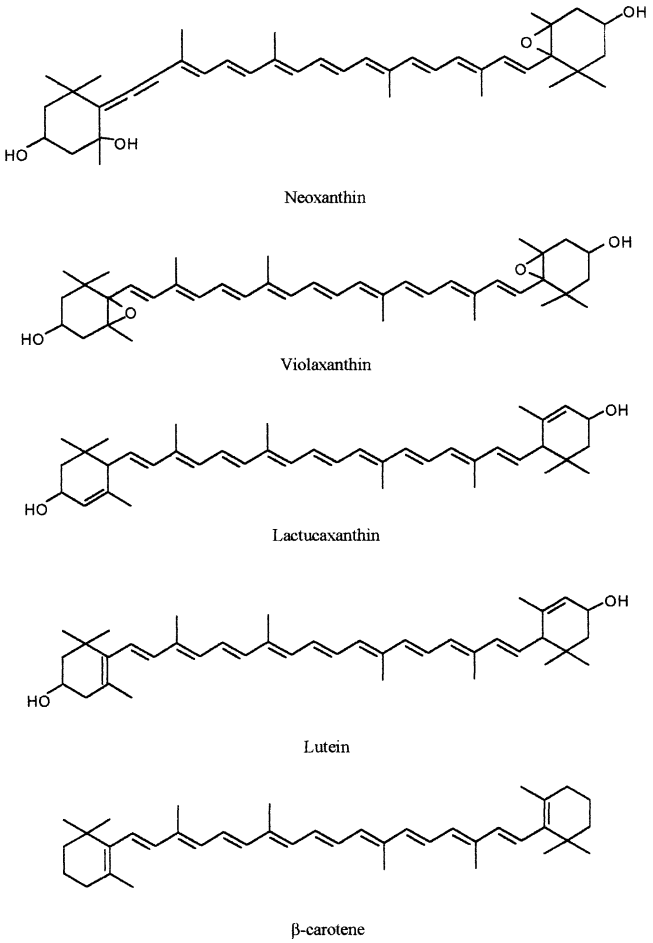


**Figure 1.** Principal carotenoids of leafy vegetables. Lactucaxanthin is found only in lettuce.

Results of the comparison of hydroponic and field-produced curly lettuce were submitted to analysis of variance and Tukey test.

## RESULTS AND DISCUSSION

**Carotenoids of the Leafy Vegetables.** The carotenoids encountered in the leafy vegetables (**Figure 1**) were identified (*10*) as follows.

Neoxanthin (5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-$\beta,\beta$-carotene-3,5,3′-triol) exhibited a visible absorption spectrum ($\lambda_{max}$ in PE = 412, 436, and 464 nm; $\lambda_{max}$ in the mobile phase = 413, 438, and 465 nm) with defined spectral fine structure (%III/II = 87), consistent with a chromophore of eight conjugated double bonds and an allenic group in the polyene chain. The presence of three hydroxyl groups and a 5,6-epoxide, indicated initially by the chromatographic behavior ($t_R$ = 8.0 min; $R_f$ = 0.02), was confirmed, respectively, by the positive response to acetylation and a hypsochromic shift of 20 nm, corresponding to the rearrangement of one 5,6-epoxide to 5,8-epoxide, on addition of dilute HCl.

Violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-$\beta,\beta$-carotene-3,3′-diol) displayed the spectrum ($\lambda_{max}$ in PE = 414, 438, and 468 nm; $\lambda_{max}$ in the mobile phase = 415, 441, and 470 nm) with well-defined fine structure (% III/II = 98) of a carotenoid with nine conjugated double bonds in the polyene chain. The chromatographic behavior ($t_R$ = 9.5 min; $R_f$ = 0.10) and positive acetylation demonstrated the presence of two hydroxyl groups. Epoxide−furanoxide rearrangement resulted in a hypsochromic shift of 40 nm, showing the presence of two epoxides at the 5,6- and 5′,6′-position.

**Table 1.** Carotenoid Composition (Micrograms per Gram)[a] of Marketed Hydroponic Leafy Vegetables Produced in Winter

| sample | Portuguese name | neoxanthin | violaxanthin | lactucaxanthin | lutein | $\beta$-carotene |
|---|---|---|---|---|---|---|
| curly lettuce | alface crespa | 6.4 ± 1.6 | 14.3 ± 3.9 | 8.2 ± 0.9 | 15.4 ± 1.6 | 17.1 ± 1.8 |
| French lettuce | alface crespinha | 10.8 ± 2.2 | 20.1 ± 2.2 | 11.9 ± 1.3 | 22.9 ± 2.6 | 24.6 ± 3.1 |
| Boston lettuce | alface lisa | 9.9 ± 1.7 | 19.2 ± 1.7 | 11.8 ± 0.7 | 21.4 ± 1.4 | 22.8 ± 1.2 |
| freelice lettuce | alface freelice | 5.4 ± 1.4 | 8.1 ± 1.1 | 6.9 ± 0.4 | 10.2 ± 1.0 | 9.9 ± 1.5 |
| roquette | rúcula | 11.5 ± 2.9 | 21.0 ± 5.9 | nd[b] | 52.2 ± 12.6 | 33.0 ± 9.9 |
| cress | agrião | 16.8 ± 3.5 | 25.9 ± 5.3 | nd | 75.4 ± 10.2 | 36.9 ± 7.0 |
| chicory | almeirão | 14.9 ± 4.8 | 20.7 ± 4.0 | nd | 57.0 ± 10.3 | 36.3 ± 7.2 |

[a] Means and standard deviations of five sample lots for each vegetable. [b] Not detected.

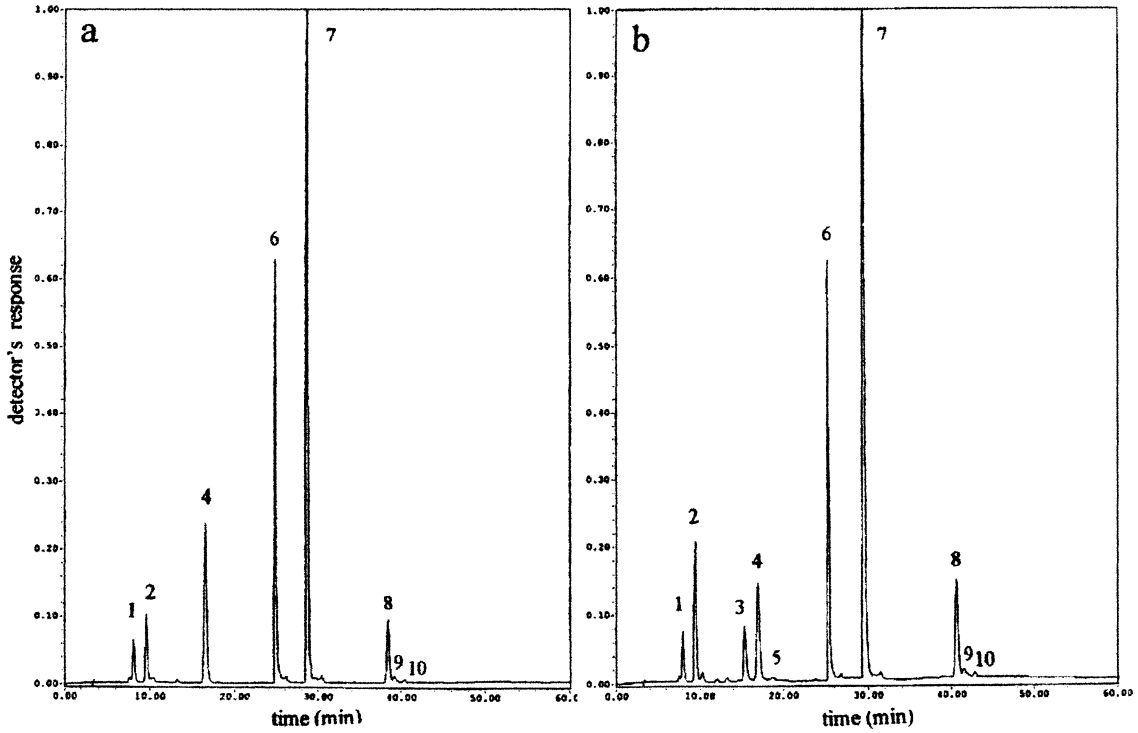


**Figure 2.** HPLC chromatograms of cress (a) and Boston lettuce (b) extracts. Peaks: 1, neoxanthin; 2, violaxanthin; 3, lactucaxanthin; 4, lutein; 5, zeaxanthin; 6 and 7, chlorophylls; 8, *trans*-$\beta$-carotene; 9 and 10, *cis*-$\beta$-carotene. HPLC conditions are described in the text.

**Table 2.** Comparison of the Carotenoid Composition (Micrograms per Gram)[a] of Hydroponic and Conventionally Produced Curly Lettuce

| sampling | production | neoxanthin | violaxanthin | lactucaxanthin | lutein | $\beta$-carotene |
|---|---|---|---|---|---|---|
| 1 | conventional | 5.8 ± 0.4a | 16.1 ± 0.9a | 7.0 ± 0.6a | 15.0 ± 0.4a | 17.6 ± 1.2a |
| | hydroponic | 4.5 ± 0.5b | 11.2 ± 0.5b | 6.1 ± 0.7a | 11.2 ± 0.2b | 12.1 ± 0.8b |
| 2 | conventional | 6.7 ± 0.5a | 22.6 ± 1.8a | 7.4 ± 0.3a | 20.0 ± 0.1a | 23.2 ± 1.3a |
| | hydroponic | 5.3 ± 0.3b | 18.1 ± 0.6b | 6.2 ± 1.2a | 16.8 ± 0.9b | 18.8 ± 0.6b |
| 3 | conventional | 6.6 ± 0.4a | 17.5 ± 0.7a | 7.7 ± 0.5a | 15.6 ± 0.5a | 18.2 ± 0.8a |
| | hydroponic | 5.1 ± 0.3b | 15.4 ± 0.5b | 6.9 ± 0.8a | 13.1 ± 0.4b | 15.9 ± 0.5b |

[a] Each value is the mean and standard deviation of three sample lots. Values in the same column for the same sampling with different letters are significantly different at $p < 0.05$.

Lactucaxanthin ($\epsilon,\epsilon$-carotene-3,3′-diol) presented a spectrum ($\lambda_{max}$ in PE = 414, 436, and 466 nm; $\lambda_{max}$ in the mobile phase = 415, 439, and 468 nm) with well-defined fine structure (%III/II = 97%) reflecting a chromophore of nine conjugated double bonds, all in the polyene chain. The positive response to acetylation confirmed the presence of two hydroxyl substituents, initially indicated by the chromatographic behavior ($t_R$ = 15.5 min; $R_f$ = 0.13). The allylic position of both hydroxyls was demonstrated by the positive reaction to methylation ($R_f$ of product = 0.98).

Lutein ($\beta,\epsilon$-carotene-3,3′-diol) had a visible spectrum ($\lambda_{max}$ in PE = 420, 442, and 470 nm; $\lambda_{max}$ in the mobile phase = 423, 447, and 475 nm) with less defined fine structure (%III/II = 60) commensurate with a chromophore of 10 conjugated double bonds, 9 in the polyene chain and 1 in a $\beta$-ring. The presence of two hydroxyl groups was shown by the chromatographic behavior ($t_R$ = 17.0 min; $R_f$ = 0.15) and the positive reaction to acetylation, and the allylic position of one of them was indicated by the positive response to methylation, producing a monohydroxylated carotenoid ($R_f$ = 0.47).

$\beta$-Carotene ($\beta,\beta$-carotene) had the typical spectrum ($\lambda_{max}$ in PE = 448 and 475 nm and a shoulder at 424 nm; $\lambda_{max}$ in the mobile phase = 454 and 480 nm and a shoulder at 428 nm) of a carotenoid with 11 conjugated double bonds, 2 of which were

located in β-rings, thus having little spectral fine structure. The absence of functional groups was reflected by the chromatographic behavior ($t_R$ = 41.0 min; $R_f$ = 0.98).

The above results agree with the finding that, unlike fruits which differ in the carotenoid composition qualitatively and quantitatively, leafy vegetables have a strikingly constant carotenoid distribution, the principal carotenoids being lutein, β-carotene, violaxanthin, and neoxanthin (*2*). Lettuce is an exception in that it also contains lactucaxanthin as a major carotenoid. Lactucaxanthin was first reported by Siefermann-Harms et al. (*12*), found specifically in lettuce. The structure was elucidated by mass spectrometry and nuclear magnetic resonance spectroscopy.

**Carotenoid Composition of Marketed Hydroponic Leaves.** The compositions of four types of lettuce, roquette, cress, and chicory are presented in **Table 1**. Typical chromatograms of the carotenoids of cress (without lactucaxanthin) and Boston lettuce (with lactucaxanthin) are shown in **Figure 2**. All of the lettuce samples had lactucaxanthin, and this is the first report on the quantitative analysis of this carotenoid. The biological significance of lactucaxanthin is not known at the moment.

Of the leafy vegetables analyzed, cress had the highest concentrations of β-carotene, lutein, violaxanthin, and neoxanthin. The lettuce freelice presented the lowest levels of these principal carotenoids. Lutein predominated in roquette, cress, and chicory, whereas β-carotene was at levels slightly higher than or equal to those of lutein in the lactucaxanthin-containing lettuce varieties. As expected, lutein and β-carotene were followed by violaxanthin and then neoxanthin, quantitatively.

It is not clear at which point of the biosynthetic pathway lactucaxanthin is formed. The pathway, accepted for so many years and now confirmed by the cloning of the genes for more than 20 different carotenogenic enzymes (*13*), branches after the formation of lycopene because cyclization to form β-carotene, which has two β-rings, occurs separately from cyclization to form α-carotene, which has one β-ring and one ε-ring. There is apparently no interconversion between β-carotene and α-carotene or between a β-ring and an ε-ring Lactucaxanthin has two ε-rings. In any case, the formation of lactucaxanthin appears to affect the production of lutein. Considering the relative ratio of lutein and β-carotene, which is fairly constant in leafy vegetables, lettuce has a lower level of lutein than the other leaves, by an amount equivalent to the lactucaxanthin content (**Table 1**).

In a previous paper (*14*), the β-carotene contents of conventionally produced curly lettuce, Boston lettuce, roquette, cress, and chicory, analyzed at different times during the year, were 14.5 ± 4.7 μg/g ($n$ = 14), 12.6 ± 5.2 μg/g ($n$ = 6), 34.6 ± 13.2 μg/g ($n$ = 5), 41.5 ± 10.0 μg/g ($n$ = 5), and 34.3 ± 9.7 μg/g ($n$ = 10), respectively. Except for Boston lettuce, which had lower β-carotene concentration, the results agree well with those of the present study, although the previous data were obtained by open column chromatography and reflected variations during the year, having higher standard deviations.

No comparison can be made in terms of the other carotenoids because data are not available for violaxanthin and neoxanthin, whereas the lutein levels were underestimated in the previous study because saponification was carried out. After a thorough investigation of the consequences of the saponification step, under different conditions (*15*) and considering that chlorophylls can be separated from the carotenoids during chromatography, this step was deleted from the analytical procedure for leafy vegetables.

**Comparison of Hydroponic and Conventionally Produced Lettuce.** A direct comparison of the carotenoid composition of conventionally produced and hydroponic curly lettuce, collected from neighboring farms, was also carried out. The conventionally produced lettuce had significantly higher β-carotene, lutein, violaxanthin, and neoxanthin levels than the hydroponic lettuce (**Table 2**).

Two processes occur in photosynthetic tissues that have opposite effects on the carotenoid content: enhancement of biosynthesis and photodegradation. Both of these processes are affected by enviromental factors, particularly exposure to sunlight and temperature.

The hydroponic farm from which the samples were taken was covered by a polyethylene roof during the whole year. This controls the amount of sunlight and the temperature to which the vegetables are exposed, which can serve as a protection against photodegradation during the summer. During the winter, the plastic covering may limit exposure to sunlight and lower the temperature to the extent that carotenoid biosynthesis is not stimulated as in vegetables in open fields, explaining the lower carotenoid values of the hydroponic lettuces analyzed in the present study. In a previous investigation, also carried out in the winter, hydroponic curly lettuces harvested in warmer periods presented higher carotenoid concentration than those collected from the same farm on colder days (*16*). In a concurrent study (unpublished data), in which minimally processed endive, kale, and spinach grown in plots (in soil) protected by polyethylene roofs were analyzed, the carotenoid levels were higher in the summer than in the winter. On the other hand, conventionally produced, field-grown leaves had been found to have a higher carotenoid content in the winter than in the summer (*17*, *18*), during which photodegration of carotenoid could prevail. Tomatoes grown in winter in greenhouses had only one-third of the total carotenoid content of outdoor produce (*19*).

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