Article

Effect of Sulfur and Nitrogen Fertilization on the Content of Nutritionally Relevant Carotenoids in Spinach (*Spinacia oleracea*)

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ABSTRACT: Spinach is an important dietary source of lutein and β-carotene. Their synthesis is closely linked to chlorophyll synthesis and dependent upon an adequate supply of sulfur and nitrogen. Soils may become sulfur-deficient during winter because microorganisms convert atmospheric SO_2 less efficiently to sulfate. The influence of sulfur and nitrogen fertilization on the carotenoid and chlorophyll contents of spinach grown in summer or winter was investigated. Carotenoid and chlorophyll levels were positively correlated. Lutein and β-carotene were 25% higher in summer than in winter. Winter levels were increased by 35−40% by sulfur fertilization in one location but not in the other, with the impact depending upon soil type, growing location, and atmospheric conditions. Carotenoids were little or not affected by nitrogen addition in winter or sulfur addition in summer. It is concluded that sulfur fertilization of spinach in winter may modestly increase carotenoids but high carotenoid levels are best assured with carotenoid-rich cultivars grown in summer.

KEYWORDS: *Lutein, β-carotene, chlorophyll, green leafy vegetables, age-related macular degeneration, pro-vitamin A*

INTRODUCTION

Over the last 30 years, sulfur (S) emissions in western Europe have been drastically decreased from 60 to 10 kg of S/ha in compliance with the Geneva Convention on Long-Range Transboundary Air Pollution.[1] Because atmospheric S in rainwater is the major S supply for plants, its reduction could adversely affect S-sensitive crops, especially plants from the Brasssicaceae, Chenopodeaceae, Liliaceae, and Fabaceae families.

Plants assimilate S from the soil mainly as sulfates (SO_4^{2-}),[2] and microorganisms are involved in the oxidation process in the soil, which converts SO_2 to SO_4^{2-}. This process is influenced by the soil nutrient balance, soil temperature, water stress, and type of soil.[3] S deficiency is difficult to distinguish visually from nitrogen (N) deficiency because both nutrient deficiencies lead to yellow leaves and reduced plant growth,[4,5] caused by chlorophyll deficiency and a disordered protein metabolism. N is essential for chlorophyll production, and deficiency results in chlorosis.[6] Even though S does not appear in the chemical structure of chlorophylls, chloroplast membranes contain sulfolipids and a S deficiency is thought to inhibit thylakoid membrane synthesis, resulting in chlorophyll deficiency.[7]

In green leafy vegetables, carotenoid concentration correlates with the level of chlorophylls. Strong positive correlations between carotenoids and chlorophylls have been reported for Swiss chard,[8] lettuce genotypes,[9] *Brassica oleracea* cultigens,[10] and a variety of further green vegetables of the Asteraceae, Brassicaceae, Chenopodeaceae, and Liliaceae families.[11] Genomic analyses have indicated a connection between chlorophyll and carotenoid biosynthetic pathways.[6] It has been reported that signaling functions of intermediate compounds of chlorophyll biosynthesis regulate the transcription of light-harvesting chlorophyll-binding proteins and

that these proteins are also responsible for carotene and xanthophyll binding.[6,12]

Carotenoids are fat-soluble pigments with yellow to orange colorations, which might be covered by the blue and green color of chlorophylls *a* and *b*. In green leafy vegetables, the major carotenoids are the xanthophyll lutein and the oxygen-free β-carotene. Carotenoids act as accessory photosynthetic pigments, with the main functions of light-harvesting and excess energy dissipation.[13] In addition, carotenoids are capable of quenching singlet oxygen (^1O) and triplet excited chlorophyll, which is produced when light intensity exceeds the photosynthetic capacity.[13]

In human nutrition, the consumption of plants rich in carotenoids has been associated with providing health benefits by reducing the risk of specific cancers, cardiovascular diseases, and chronic eye diseases.[14] Dark green leafy vegetables are high in lutein and β-carotene and are frequently consumed in Switzerland and other European countries. Spinach is one of the vegetables with the highest lutein and β-carotene concentrations,[15] and periodic spinach consumption has been reported to increase plasma lutein concentration and to increase the macular pigment (MP) density in the retina.[16−18] In the study by Kopsell et al.,[18] however, a significant increase in both parameters was only observed when spinach cultivars high in lutein were consumed. The lutein and β-carotene contents of spinach are dependent upon the plant genetics but can also be strongly influenced by environmental conditions and pre- and postharvest factors.

The influence of S deficiency on the carotenoid content of spinach has not been documented; however, the yellow coloration of winter spinach compared to summer spinach (as frequently observed at ACW) could be due to S deficiency. In this paper, the results of three field trials designed to investigate the effects of S and N fertilization on carotenoid and chlorophyll concentrations in spinach are reported. The aims of the trials were to investigate the influence of different levels of S fertilization in combination with the season (winter versus summer, trials 1 and 2) and to test additional N fertilization rates (trial 3) on spinach yield and carotenoid and chlorophyll concentrations.

■ MATERIALS AND METHODS

Spinach Cultivation. *S Fertilization Winter and Summer (Trial 1 and 2).* Winter and summer spinach (*Spinacia oleracea* cv. 'Clermont') were seeded in October 2009 and August 2010, respectively, at the Research Station ACW in Wädenswil (Switzerland). For both S fertilization trials, the field was treated with 2 kg/ha of 'Venzar' herbicide (80% Lenacil; Bayer CropScience, Zollikofen, Switzerland) directly after seeding. The study design for both trials was identical and consisted of one S-untreated group (negative control) and five S-treated groups with progressively increasing rates of S addition (10, 20, 40, 60, and 80 kg of S/ha). Each of the six treatments was randomly assigned to four repetition plots of 1.5 × 12 m within the field, and fertilizers were applied as described below. All fertilizers were purchased from Landor (Birsfelden, Switzerland).

For the winter spinach trial 1, the first application of nutrients for all treatments was carried out at the end of February 2010. For the S-free control treatment, this consisted of 30 kg of P_2O_5/ha as 'triplesuperphosphat' (20.1% P and 12% Ca), 30 kg of N/ha in the form of calcium nitrate (15.5% N), and 240 kg of K_2O/ha in the form of potassium chloride (48% K). For the treatments with increasing amounts of S, potassium sulfate (41.5% K and 18% S) was increased at the expense of potassium chloride. For the winter spinach trial 1, a total of 150 kg of N/ha was applied at three different stages of crop development: the first application of 30 kg of N/ha as calcium nitrate (15.5% N) was applied at the end of February 2010, as described above, a second application of 90 kg of N/ha in the form of calcium ammonium nitrate (27% N) was applied 20 days later, and the final application with 30 kg of N/ha as calcium nitrate (15.5% N) was made after an additional 20 days. The spinach samples were harvested at the beginning of May 2010.

For the summer spinach trial 2, fertilization with phosphorus, potassium, and sulfur was identical to that used for the winter spinach trial 1 and these nutrients were applied at the beginning of August 2010. The amount of N fertilizer applied (150 kg of N/ha) was also identical but was divided into only two applications of calcium ammonium nitrate (27% N). The first application of 90 kg of N/ha was made shortly before sowing, and the second application of 60 kg of N/ha was carried out 20 days later (end of August). The spinach samples were harvested at the end of September 2010.

Combined S and N Fertilization in Winter (Trial 3). In October 2010, the winter spinach trial 1, as described above, was repeated in the St. Galler Rheintal (Switzerland) with the identical spinach cultivar 'Clermont' and the same six levels of S but additionally with three different levels of N fertilization (150, 180, and 210 kg of N/ha). As in the previous trials, all treatments were randomly assigned to four different field plots. The size of the plots in trial 3 was 4.5 × 4.5 m. In total, there were 72 single plots (6 S levels × 3 N levels × 4 repetitions). The basic fertilization levels were identical to trials 1 and 2 and consisted of 30 kg of P_2O_5/ha as 'triplesuperphosphat' (20.1% P_2O_5 and 12% Ca) and 240 kg of K_2O/ha, which was applied as different amounts of potassium chloride (48% K) and potassium sulfate (41.5% K and 18% S) depending upon the S treatment level. The different quantities of N fertilizer were each applied in the form of calcium nitrate (15.5% N) over three separate applications: the first in early February, then in early March, and finally, in late March 2011.

For each treatment, the amount of N applied was identical at each time point. Spinach samples were harvested at the end of April 2011.

Methods. *Sample Preparation.* At harvest, a 1 m² area of spinach, in the middle part of each plot, was collected as a representative sample of each treatment and repetition. Each sample was weighted at the field site as a measure of crop yield. Inedible leaves were then removed. Samples were washed with cold water and dripped in a commercial salad centrifuge. Finally, other inedible parts were removed with a kitchen knife. Spinach samples were stored at 1 °C and further prepared within 6 h of harvest. A representative amount (5−7 kg) of spinach from each of the four treatment plots was mixed in a box. An aliquot of 500−750 g was flushed with liquid N_2 (Messer Schweiz AG, Lenzburg, Switzerland) and ground to a fine powder at 1 °C with a cutter (La Moulinette DPA 1, Germany). Finally, the frozen spinach powder was stored at −20 °C in amber-colored bottles until extraction.

Extraction. The extraction solvent consisted of a 1:1 (v/v) mixture of methanol and acetone (Acros Organics Chemie Brunschwig, Basel, Switzerland), with a purity >99%, with an additional 50 μL/L of 2,6-di-*tert*-buthyl-methylphenol (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). Aliquots (5 g) of the frozen powder were mixed with 60 mL of extraction solvent, flushed with N_2 for 30 s, and then homogenized for another 30 s with a Polytron (Polytron PT 3100, Merck, Zug, Switzerland) at maximum speed. The extracts were made up to 100 mL with additional extraction solvent and allowed to stand for 5 min for sedimentation.

For high-performance liquid chromatography (HPLC) analyses, an aliquot of the supernatant was filtered through 0.45 μm nylon filters (OPTI-Flow, WICOM, Heppenheim, Germany) directly into a HPLC vial. The extraction of each treatment plot repetition was performed in duplicate.

Reference Compounds. Lutein (purity of 96%) was purchased from Carotenature (Lupsingen, Switzerland), and *β*-carotene (purity of 97%) as well as chlorophylls *a* and *b* were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland).

Chromatographic Analysis of Carotenoids and Chlorophylls. Carotenoid and chlorophyll analysis was carried out in a single run using a Varian HPLC system with diode array detection (DAD) (Varian ProStar 335, Australia) fitted with a C30 column (YMC C30, 5 μm, 4.3 × 250 mm; YMC Europe GmbH, Dinslaken, Germany) and operated at room temperature. The injection volume was 50 μL, and elution was completed in 36 min with a methanol/methyl-*tert*-butyl-ether (MTBE) gradient, both with a purity >99% (Fisher Scientific, Wohlen, Switzerland) and a flow rate of 1.0 mL/min.

Individual carotenoid and chlorophyll identification was based on the spectrum and the retention time of the reference compounds. Quantification was performed by means of external standard calibrations at 450 nm for the carotenoids and 650 nm for the chlorophylls. The limit of quantification was determined to be 0.05 mg/100 g of fresh matter (FM). Single-field plot repetitions were extracted in duplicate, which resulted in mean standard deviations of ≤5% for each carotenoid and chlorophyll measured.

Statistical Analysis. Values in Figures 1 and 2 and Table 1 represent the means of four field repetitions. All statistical analyses were conducted with XLSTAT Pro Version 2011.2.04 (Addinsoft, Andernach, Germany) using analysis of variance (ANOVA) to evaluate significant differences. Multiple pairwise comparisons were evaluated by means of Tukey's (HDS) post-hoc test.

■ RESULTS AND DISCUSSION

The different treatments used in the three trials resulted in a range of carotenoid contents, with the higher carotenoid contents being double the lower levels. The lutein and *β*-carotene contents of the spinach cultivar 'Clermont' ranged from 4.2−8.4 mg/100 g of FM and 3.3−7.2 mg/100 g of FM, respectively. The higher levels observed in the present study are similar to those reported by Kopsell et al.,[18] who found mean lutein and *β*-carotene concentrations of 8.0−10.0 mg/100 g of

FM and 5.3−7.9 mg/100 g of FM, respectively, in 13 spinach cultigens grown over 2 years. Hart and Scott[19] reported lutein and β-carotene contents of 5.9 mg/100 g of FM and 3.4 mg/100 g of FM, respectively, in non-specified spinach cultivars.

Influence of Season and S Fertilization (Trials 1 and 2). In the S fertilization trials 1 and 2, the levels of lutein and β-carotene were higher in summer spinach than in winter spinach (Figure 1). Growing spinach without S fertilization in summer
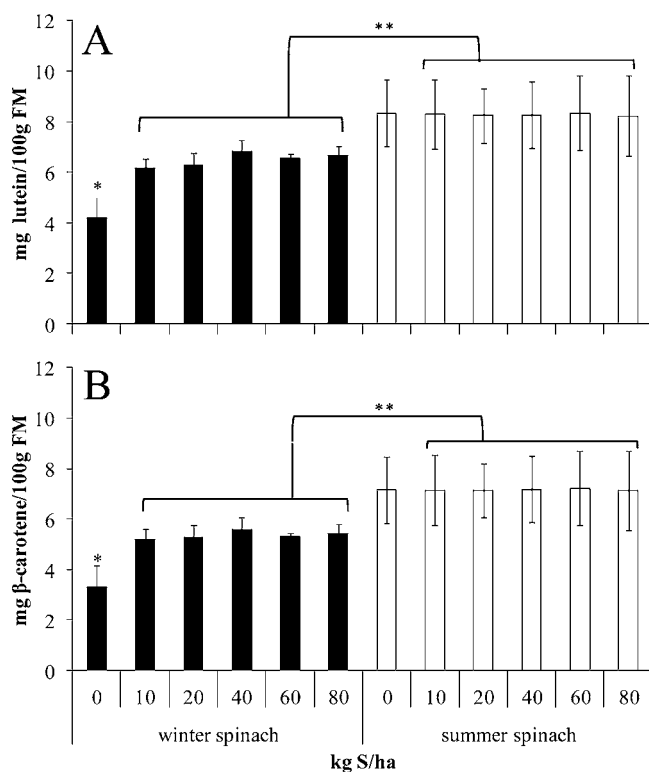

Figure 1. Influence of S fertilization on (A) lutein and (B) β-carotene concentrations (mg/100 g of FM) in winter (■) and summer (□) spinach harvested in 2010. Bars show the mean of four repetitions. (∗) $p < 0.001$ indicates a significant difference within winter spinach. (∗∗) $p < 0.01$ indicates a significant difference between S-treated winter and summer spinach.

as opposed to winter resulted in an approximate 2-fold increase in both carotenoids ($p < 0.0001$). Adding S had no influence on the carotenoid content of summer spinach ($p > 0.05$) but increased the lutein and β-carotene concentrations in the winter spinach by 35−40% ($p < 0.001$) with no significant differences between the different rates of S application ($p > 0.05$). However, even with the S fertilization, the carotenoid levels were still lower in the winter for the five S-treated groups, with 22% less lutein ($p = 0.007$) and 25% less β-carotene ($p < 0.001$) compared to summer spinach. The lack of S could thus be a reason for lower carotenoid contents in the winter. However, other factors, such as the longer growing season with lower temperatures and less light, undoubtedly play an important role. Carotenoid synthesis is a light-dependent process, and plants alter the carotenoid accumulation and composition in response to light.[20]

The negative control of winter spinach 2010, which responded to S treatment, showed the typical characteristics of chlorosis with yellow leaves because of chlorophyll deficiency.[4] Providing S through fertilization returned the plants to their typical green color. In higher plants without

mineral deficiencies, carotenoids are masked by the presence of chlorophylls. Carotenoids accumulate in the photosynthetic thylakoid membranes of green leaves, where they are bound to specific chlorophyll/carotenoid-binding protein complexes of the two photosystems.[20] S deficiency in the control spinach of winter 2010 could be explained by a lower soil sulfate (SO_4^{2-}) content, because of a decreased transformation of SO_2 to sulfate by the soil microorganisms at soil temperatures less than 10 °C[21] and an increased SO_4^{2-} leaching as a result of more rain in that year. During the winter trial 1 in 2010, the soil temperature (T_{soil} of 10 cm) throughout the growing period was below 10 °C for approximately 60% of the time (data provided by MeteoSchweiz). Because no differences have been observed between the S treatments (10−80 kg of S/ha), a moderate S fertilization of 10 kg/ha seems to be sufficient for optimal carotenoid and chlorophyll concentrations for winter spinach under given environmental conditions.

The results from trial 2, where no influence of S fertilization was observed, are consistent with data from Kopsell et al.,[6] who reported no influence of increasing S rates on lutein, β-carotene, and chlorophyll contents in watercress. Other workers have evaluated the influence of seasons on the carotenoid content of leafy vegetables with varying results. Mou[9] found small but statistically significant differences for lutein and β-carotene between summer and autumn planting of an assortment of lettuce cultigens. The mean carotenoid content of the summer planting was 7% higher ($p < 0.05$) than the autumn planting; however, in contrast to the present study, their plants were sown and harvested in autumn and there would presumably be less distinctive meteorological differences between summer and autumn than between summer and winter. The daily temperatures were higher in summer than in autumn but did not fall below 10 °C during either period. The solar radiation reported for autumn was lower compared to summer, which could explain the slightly decreased carotenoid concentrations in the autumn trial.[9] Mercadante and Rodriguez-Amaya[22] compared carotenoids in two different kale cultivars grown in summer or winter. In one cultivar, they found no differences, whereas in the other cultivar, they reported a 32% significantly lower lutein content ($p = 0.0002$) and a 22% significantly lower β-carotene content ($p = 0.0001$) in summer. This is in direct contrast to results presented here, and the authors concluded that light exposure and temperatures during the summer may have exceeded the optimum conditions for carotenoid synthesis and might have conversely caused carotenoid destruction. It appears therefore that seasonal factors, such as solar radiation and temperature, influence the carotenoid concentrations of green leafy vegetables differently depending upon the botanical family and cultivar.

Influence of Year and Growing Location (Trials 1 and 3). Rather surprisingly, in the winter trial 3, S fertilization of spinach failed to increase the carotenoid content (Figure 2) as it had done in winter trial 1 (Figure 1). In this study, the control spinach without S addition maintained its green color and had similar concentrations of carotenoids as the S fertilized spinach. The two trials were carried out at different locations with different types of soil. The soil in Wädenswil for trial 1 was richer in loam and should have had a better capacity for retaining water and water-soluble sulfates than the soil in trial 3 in the St. Galler Rheintal, which had a higher proportion of sand, normally facilitating the leaching of sulfates. The rainfall during the growing period of trial 1 in 2010 averaged 3 mm/day and was significantly higher than the 0.8 mm/day in trial 3
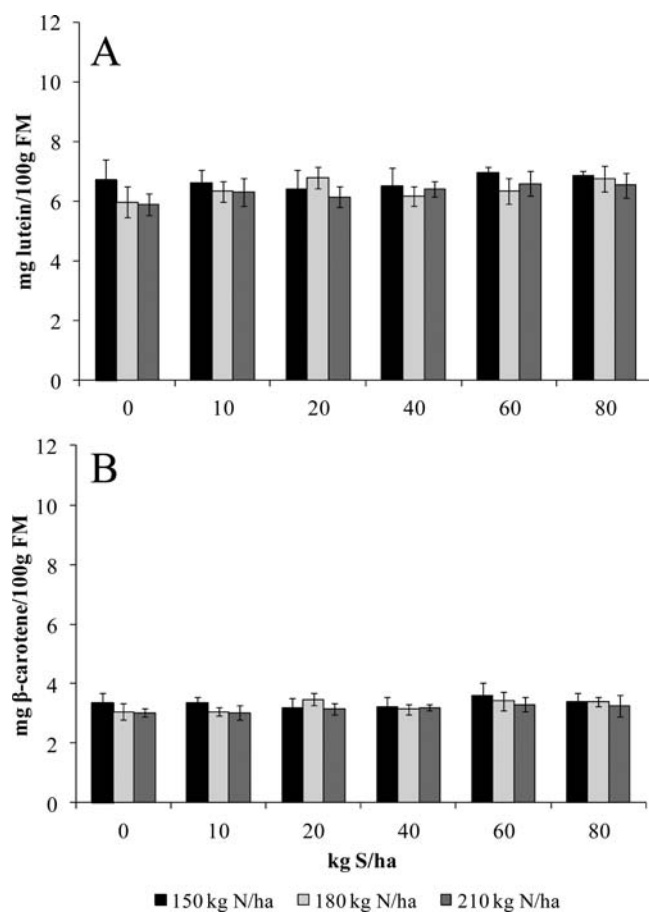
Figure 2. Influence of S and N fertilization on (A) lutein and (B) β-carotene concentrations (mg/100 g of FM) in winter spinach harvested in 2011. Bars show the mean of four repetitions. No significant differences ($p < 0.05$) could be observed.

made in 2011. It would appear therefore in trial 1 that the leaching of sulfate through excessive rainwater exceeded the sulfate retaining capacity of the loam soil, whereas the much lower precipitation in trial 3 onto the sandy soil resulted in less sulfate leaching. Therefore, no sulfur deficiency symptoms and higher carotenoid levels have been observed.

When averaging the carotenoid and chlorophyll contents of all treatment groups in trials 1 and 3 with identical N fertilization levels but different levels of S fertilization, there were significant differences between the two trials in the mean pigment contents. Lutein ($p = 0.003$) and chlorophyll b ($p = 0.018$) concentrations were significantly higher in trial 3, whereas β-carotene ($p < 0.001$) and chlorophyll a ($p = 0.018$) contents were significantly higher in trial 1. The mean lutein content was 6.1 mg/100 g of FM in trial 1 compared to 6.7 mg/100 g of FM in trial 3, whereas the β-carotene concentration was 5.0 mg/100 g of FM in trial 1 and 3.4 mg/100 g of FM in trial 3, respectively. Year-dependent differences in carotenoid accumulation have also been reported by Kopsell et al. for spinach[18] and kale.[10] The mean lutein and β-carotene concentrations in 13 spinach cultivars decreased from 10 and 7.9 mg/100 g of FM in 1 year to 8.0 and 5.3 mg/100 g of FM in the following year.[18] In the kale study, the mean lutein and β-carotene values of 23 kale cultigens were 6.8 and 5.2 mg/100 g of FM, respectively, in the first growing year and 8.1 and 6.3 mg/100 g of FM, respectively, in the second year.[10] In contrast to the present investigation, Kopsell et al.[18] used the

same growing site for spinach for both years and observed a parallel decrease in the lutein and β-carotene concentrations from the first to the second year. The fact that lutein increased and β-carotene decreased over the 2 years in the present study suggests that the carotenoid content of green leafy vegetables depends upon several factors relative to growing site and year, including type of soil, solar radiation, and other atmospheric, environmental, and agricultural conditions.

The ratio of lutein/β-carotene in the winter spinach was 1.2:1 in 2010 and 2:1 in 2011. Kopsell et al.[18] found less variation between the relative amounts of carotenoids over the two growing years and reported ratios of 1.3:1 and 1.5:1. β-Carotene is mainly located in the core region of photosystem I,[23] and lutein is mainly located in the core region of photosystem II.[20] A higher sunlight exposure is reported to increase the leaf xanthophylls but negatively affect β-carotene concentrations.[20] Therefore, it is possible that a higher solar radiation in trial 3 resulted in a higher lutein/β-carotene ratio.

The ratio of total carotenoids (lutein + β-carotene) and total chlorophylls (chlorophylls a + b) appears to be much more stable and was 1:12 in 2010 (winter and summer) and 1:9 in 2011 (Figure 3). Lefsrud et al.[24] have previously reported a
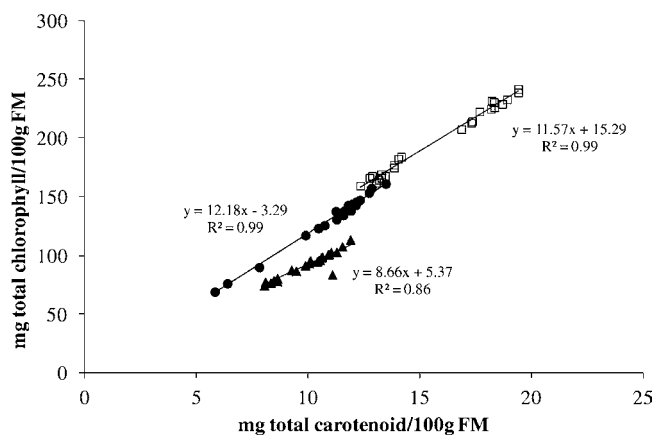

Figure 3. Correlation between the sum of lutein and β-carotene (total carotenoid) and the sum of chlorophyll a and b (total chlorophyll) contents in winter 2010 (●), summer 2010 (□), and winter 2011 (▲) spinach. Symbols show the mean of four repetitions of all sulfur fertilization rates with moderate nitrogen fertilization (150 kg of N/ha).

similar ratio of 1:11 for carotenoid and chlorophyll concentrations of spinach grown under different irradiation levels and found a significant influence on carotenoid concentrations (on the basis of dry matter). In the presented studies, no differences in the chlorophyll/carotenoid ratio between the seasons were observed; however, a strong influence of growing site and year was observed. The growing years are characterized by environmental conditions, including temperature and solar radiation, which strongly influence pigment accumulation and relationships in green leafy vegetables.

In addition to the pigment concentrations in spinach, yields were shown to be unaffected by S treatments in both years (Table 1). There was a tendency of a slightly decreased yield in the negative control of trial 1, which developed the yellow coloration. However, the high variance in yield values for the different field repetitions could be responsible for the failure of statistical significance. Samatanova et al.[25] have previously

Table 1. Mean Yield (kg/m²) and Standard Deviation of Four Field Repetitions of Winter Spinach 2010 and 2011 with a N Fertilization Rate of 150 kg/ha[a]

S fertilization rate (kg of S/ha)	2010 yield (kg/m²)	2011 yield (kg/m²)
0	1.9 ± 0.9	2.5 ± 0.6
10	2.7 ± 0.4	2.3 ± 0.7
20	3.0 ± 0.8	2.3 ± 0.5
40	2.7 ± 0.7	2.6 ± 0.3
60	2.7 ± 0.6	2.3 ± 0.2
80	3.0 ± 0.6	2.2 ± 0.3

[a]No significant differences ($p < 0.05$) were observed.

reported a significantly lower yield in S-deficient spinach compared to S-treated groups. This trial was carried out in plastic pots containing exactly 12 seeds,[25] which is a more precise method to assess yield than cutting out 1 m² spinach from the central row of the field. Other studies have additionally reported no influence of S fertilization rates on the shoot fresh matter or dry matter for kale[5] and vegetables of the *Brassica* genus.[26,27]

Influence of Combined Sulfur and Nitrogen Fertilization (Trial 3). As reported before, S fertilization had no influence on the lutein and β-carotene concentrations in spinach (Figure 2). Increasing rates of N fertilization also caused only small changes in carotenoids. The mean of the lowest N fertilization rate (150 kg/ha) without consideration of S treatment showed slightly higher pigment concentrations in comparison to 180 and 210 kg of N/ha, but this was statistically significant only for lutein ($p = 0.036$) with 6.7 mg/100 g of FM (150 kg of N/ha) and 6.4 mg/100 g of FM (180 and 210 kg of N/ha), respectively. Kopsell et al.[28] found no significant effect of increasing N on carotenoid pigments in kale but similarly observed the tendency of reduced carotenoid accumulation with increasing nitrogen fertilization. In contrast, the carotenoid content of watercress increased linearly with an increasing N fertilization rate.[6] Likewise, the carotenoid and chlorophyll contents of parsley responded to N fertilization, and the highest pigment concentrations were found in parsley treated with the highest N rate.[29] As indicated in the review by Mozafar,[30] the influence of N fertilization on pigment contents thus appears to be vegetable-specific.

As might be expected from the above discussion, the combination of N and S fertilization also showed no clear impact on pigment content in winter spinach. Although the combination of the lowest N (150 kg/ha) and highest S (60 and 80 kg/ha) treatment resulted in slightly higher carotenoid and chlorophyll contents, no statistically significant interactions between N and S treatments were observed. These findings are in accordance with Kopsell et al.,[6] who reported that pigment contents of watercress were unaffected by the combined N and S treatment.[6]

Nutritional Aspects of Spinach Consumption. Among vegetables, spinach has one of the highest lutein and β-carotene concentrations.[11,15] It is frequently consumed in Europe[31] and provides almost a third of the daily lutein intake in Spain, France, The Netherlands,[31] and Switzerland.[11] An increase in the carotenoid levels in spinach and other green leafy vegetables over the summer season would increase lutein and β-carotene intakes. Both carotenoids have important physiological functions in humans. Because β-carotene is a vitamin A precursor, the intake of this carotenoid can support epithelial cell differentiation and visual functions in the rhodopsin cycle.

Lutein is also present in the retina, with its highest density in the macula, where it prevents the retina and the retinal pigment epithelium from light-induced oxidative damage.[16] In addition to these specific well-accepted functions, both carotenoids are reported to act as radical scavengers, enhancers of immune responses, and promoters of cell communication.[14]

The influence of a periodic consumption of spinach with different contents of lutein and β-carotene was investigated by Kopsell et al.[18] The two spinach cultivars fed to adult volunteers contained 12.1 or 8.4 mg of lutein and 9.2 or 6.5 mg of β-carotene/100 g of FM, respectively. After 12 weeks of feeding two separate groups of 10 volunteers with five 50 g portions of cooked spinach/week, serum lutein concentrations increased in both groups. However, the 33% increase in the high lutein group was significantly higher ($p = 0.038$), whereas the 22% increase in the lower lutein group failed to reach statistical significance ($p = 0.07$). In addition, MP density in the retina only improved significantly in the high lutein group ($p = 0.021$). Rather surprisingly, a decreased serum β-carotene concentration was observed in both intervention groups,[18] which could be explained by a less effective release of carotenes from the leaf matrix caused by their low polarity and the competition between lutein and β-carotene during the micellar absorption process. Other intervention studies with cooked spinach have similarly reported an increase in MP density and higher serum carotenoid levels, with an increased serum lutein concentration in the majority of subjects.[16,17]

High carotenoid intake can be assured by choosing a carotenoid-rich spinach cultivar; however, a further increase in levels might be possible by modification of the preharvest factors. Growing the spinach in the summer season as opposed to the winter appears to have the greatest influence, presumably because of higher solar radiation and increased temperatures. Without S fertilization, there was a doubling of the concentration of both carotenoids. The mean lutein and β-carotene contents in summer spinach were 8.3 and 7.2 mg/100 g of FM, respectively, as opposed to 6.1 and 5.0 mg/100 g of FM in winter. S fertilization appears to have no influence on carotenoid contents in summer spinach but may have a modest influence in winter. The extent of the effect, however, is modest and difficult to predict, because it appears to depend upon soil type, soil temperature, rainfall, growing location, and other factors that influence the conversion of SO_2 to sulfate by soil microorganisms. Where soil is S-deficient in the winter period because of increased sulfate leaching or low activity of the soil bacteria, even a modest fertilization rate of 10 kg of S/ha can cause an appreciable increase in carotenoid content, with no further increases at higher levels of S fertilization.

In conclusion, the best way to ensure a high carotenoid intake from spinach is to choose a cultivar rich in carotenoids that has been grown over the summer period. S fertilization did not further increase the carotenoid content of summer spinach. The lower levels of carotenoids in winter spinach may be modestly increased with S fertilization, but achieving a positive effect seems to depend upon soil type, growing location, and atmospheric conditions. N fertilization had little or no influence on the carotenoid content of winter spinach.

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■ ABBREVIATIONS USED

FM, fresh matter; MP, macular pigment

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