

Nitrogen Concentration Affects Nutrient and Carotenoid Accumulation in Parsley

Christin H. Chenard, Dean A. Kopsell,* and David E. Kopsell

Department of Plant Biology, The University of New Hampshire, Durham, New
Hampshire, USA

ABSTRACT

Previous research has suggested that the herbal crop parsley (*Petroselinum crispum* Nym.) has a relatively high concentration of nutritionally important carotenoid phytonutrients, such as lutein-zeaxanthin, and β -carotene. Nitrogen (N) has the most direct impact on plant growth, but influence of N on phytonutritional quality is contradictory. Therefore, the objectives of this study were to measure the effects of different concentrations of N on growth, elemental accumulation, and carotenoid production in parsley. 'Dark Green Italian' parsley was greenhouse-grown in a nutrient solution with 6.0, 13.1, 26.3, 52.5, or 105.0 mg N L⁻¹. After eight weeks, plants were harvested and analyzed for biomass production, micro- and macronutrient concentrations, and lutein-zeaxanthin and β -carotene levels. Increasing N in the nutrient solution increased plant biomass, leaf tissue N, phosphorus (P), potassium (K), as well as lutein-zeaxanthin, β -carotene, and chlorophyll. Leaf iron (Fe), manganese (Mn), and molybdenum (Mo) decreased with increases in N in nutrient solutions. Quadratic increases in response to increasing solution N occurred for leaf calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), and zinc (Zn). Increasing the elemental and carotenoid concentrations in parsley through N fertility modification would be expected to increase the nutritional value of this culinary herbal crop.

Key words: β -carotene, chlorophyll, human health, lutein, zeaxanthin, macronutrient, micronutrient.

* Correspondence: Dean A. Kopsell, Department of Plant Biology, G47 Spaulding Hall, The University of New Hampshire, Durham, NH 03824-3544, USA; E-mail: kopsell@cisunix.unh.edu.

INTRODUCTION

Parsley (*Petroselinum crispum* Nym.) is a popular herbal crop valued for its aromatic and attractive leaves in cooking or in fresh consumption. Parsley leaves are used as a condiment, garnish, and flavoring ingredient.^[1] A member of the Apiaceae (Umbelliferae), parsley is a biennial, or short-lived perennial, often cropped as an annual. The distinctive flavor of parsley comes from *p*-1,3,8-menthatriene, the dominant volatile oil in the leaves.^[2] Apart from the unique flavor it possesses, parsley can also provide dietary sources of calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg), and iron (Fe), as well as vitamin A, vitamin C, and carotenoids.^[3,4]

Current fertilization recommendations for parsley production range from 56 to 196 kg nitrogen (N) ha⁻¹, 56 to 168 kg P₂O₅ ha⁻¹, and 56 to 168 kg K₂O ha⁻¹.^[5] Nitrogen has a pronounced influence on plant growth and development, and all economically important horticultural crops have recommended N rates for optimal yield. Applying N in excess of plant requirements can increase chances of N loss to the environment, therefore proper N fertility application is important for nutrient management.^[6] Mozafar^[7] reported that N fertilization increased, decreased, or had no effect on vitamin and carotene contents of several vegetable species. Because of the reported contradictions of N rate on plant quality, fertilization studies to determine optimal yield should also address chemical quality factors.^[8]

Carotenoids are a class of secondary plant compounds that act as accessory photosynthetic pigments. Plant carotenoids are divided into two groups; the xanthophylls, such as lutein and zeaxanthin, and the carotenes, such as β -carotene and α -carotene.^[9] These compounds serve many functions including light harvesting, structure stabilization, and excess energy dissipation.^[10] Additionally, they protect plants from free radicals, such as triplet excited chlorophyll (³Chl) and singlet oxygen (¹O), produced when light intensity exceeds photosynthetic capacity.^[11] Fruits and vegetables are primary sources of carotenoids in human diet, and intake of lutein and β -carotene has been associated with decreased risks of cancer and chronic disease.^[12,13]

Parsley can be a source of carotenoids, vitamins, and minerals when consumed as part of the human diet. Knowledge of growing conditions that result in the nutritional enhancement of parsley may be advantageous for production strategies. Because of the influence of N on crop growth and quality factors, the objectives of the current study were to measure the effects of different N treatment levels on: 1) plant biomass production; 2) macronutrient and micronutrient concentrations; and 3) plant pigment, especially lutein-zeaxanthin and β -carotene carotenoids, accumulation in leaf tissues of parsley.

MATERIALS AND METHODS

Plant Culture

A flat-leaf parsley cultivar, 'Dark Green Italian,' (Johnny's Selected Seed, Winslow, ME) was sown into growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) on January 17, 2003. The cells were filled with fine vermiculite and watered twice daily. The medium was supplied with bottom heat (23°C) for seed germination, and seedlings were greenhouse grown (22°C day/14°C night set points) for two weeks under natural photoperiods (lat. 43°09'N). Nutrients were applied weekly using a 200 mg L⁻¹ solution of 20 N - 6.9 P₂O₅ - 16.6 K₂O water-soluble fertilizer (Grace-Sierra, Milpitas, CA). On February 7, 2003, the seedlings were transferred to 38-L containers (Rubbermaid Inc., Wooster, OH) which held 30-L of a modified nutrient solution.^[14] Eidsten and Gislerød^[15] reported that parsley transferred to nutrient solution 3 weeks after sowing performed better than seedlings transferred 5 or 7 weeks after sowing. Fifteen parsley plants were placed into 2.2 cm holes at 10.6 cm x 9.5 cm spacing on each container lid. Elemental concentrations of the nutrient solutions were (mg L⁻¹): P (15.3); K (117.1); Ca (80.2); Mg (24.6); Fe (0.5); boron (B)(0.25); molybdenum (Mo)(0.005); copper (Cu)(0.01); manganese (Mn)(0.25); and zinc (Zn)(0.025). Plants were grown under increasing N treatment concentrations at 6, 13, 26, 52, and 105 mg N L⁻¹. The two dominant N forms were balanced in all of the N treatments to achieve a 75% NO₃⁻: 25% NH₄⁺ ratio. Solutions were aerated with an air blower (model VB-007S; Sweetwater, Ft. Collins, CO) connected to air stones. The experimental design was a randomized complete block with four replications. Nutrient solutions were replaced every 2 weeks throughout the experiment to refresh the solution to the initial nutrient concentrations.

Plants were harvested on April 4, 2003. Eidsten and Gislerød^[15] reported that parsley was saleable 6 to 8 weeks after transplanting into nutrient solution. Upon harvest, leaves from ten uniform plants in each treatment in a replication was pooled and weighed for fresh weight accumulation. Leaves were washed with soap (Aquet; Bel-art Products, Pequannock, NJ), rinsed, and blotted dry with paper towels. The parsley leaf tissue was then evenly separated into two groups. One leaf tissue group was dried at 60°C for no less than 72 h, at which time dry weight was calculated. The other group was placed in a -80°C freezer prior to lyophilization. Tissues were lyophilized for 48 h (model 6 L FreeZone; LabConCo, Kansas City, MO) prior to extraction.

Elemental Determination

Dried leaves were ground to pass a 0.5-mm screen in a sample grinder (Cyclotec Sample Mill; model 1093; Tector, Höganäs, Sweden). Ten mL concentrated nitric acid (70% HNO₃) was added to 0.3-g leaf tissue and digested in a microwave accelerated reaction system (MARS5, CEM Corp., Matthews, NC). Digestion solutions were allowed to cool to room temperature (~26°C) and adjusted to a final volume of 40 mL with deionized water. Elemental concentrations were determined by inductively coupled argon plasma –

Atomic Emission Spectrometry (ICP-AES; model Vista AX; Varian, Inc., Palo Alto, CA).^[16]

Chlorophyll and Carotenoid Determination

Tissue extraction. Freeze-dried tissues were macerated with ~50 g of dry ice in a household food chopper (Handy Chopper Plus; Black & Decker, Towson, Md.). Macerated tissues were placed in 20-ml scintillation vials, and CO₂ gas was vented prior to storage of tissues at -20°C. Samples were extracted and separated by a procedure, which is described below (G. Beecher and P. Howard, personal communication, USDA Food Composition Laboratory, Human Nutrition Research Center, Beltsville, Md.)^[17] A 0.10-g subsample was placed into a Potter-Elvehjem tissue grinder tube (Kontes, Vineland, NJ) and re-hydrated with 0.8 mL of deionized-distilled water. The sample was then placed into a water bath at 40°C for 20 min. After incubation, 0.8 mL of ethyl-β-8-apo-carotenoate (Sigma Chemical Co., St. Louis, MO), as an internal standard and 2.5 mL of tetrahydrofuran (THF) stabilized with 25 ppm 2,6-Di-*tert*-butyl-4-methoxyphenol (BHT) were added. The sample was vortexed and homogenized in the tube using ~25 insertions with a Potter-Elvehjem tissue grinder pestle attached to a drill press (Craftsman, Sears, Roebuck and Co., Hoffman Estates, IL) set at 540 rpm while the tube was immersed in ice. The tube was then placed into a clinical centrifuge for 3 min at 500 x g_n. The supernatant was removed with a Pasteur pipet, placed into a conical 15-mL test tube, capped, and held on ice during the remainder of the extraction. The sample pellet was re-suspended in 2 mL THF, and the same extraction technique was performed 3 more times until the supernatant was colorless. The remaining sample pellet was discarded, and the combined supernatants were placed in a water bath (40°C) and reduced to 0.5 mL under a nitrogen stream (model N-EVAP 111; Organomation Inc., Berlin, MA). To each 0.5 mL sample, 2.5 mL methanol, and 2 mL THF were added and vortexed. Samples were filtered through a 0.20-μm polytetrafluoroethylene filter (model Econofilter PTFE 25/20; Agilent Technologies, Wilmington, DE) prior to HPLC analysis.

High Performance Liquid Chromatography (HPLC) Analysis. An Agilent 1100 series HPLC unit with a photo diode array detector (Agilent Technologies) was used for sample separation.^[17] All samples were analyzed for chlorophyll and carotenoid compounds using a Vydac RP-18 5.0-μm 250 x 4.6-mm column (model 201TP54; Phenomenex, Torrance, CA) fitted with a 4 x 3.0-mm 7.0-μm guard column (RP-18; Phenomenex).^[17] The column was maintained at 16°C using a thermostatted column compartment. Eluents were A) 75% acetonitrile, 20% methanol, 5% hexane, 0.05% BHT, 0.013% triethylamine (TEA) in water (v/v) and B) 50% acetonitrile, 25% THF, 25% hexane, 0.013% TEA in water (v/v). The flow rate was 0.7 mL/min and the gradient is 100% A for 30 min, 50% A and 50% B for 2 min; 100% B for 2 min; and 50% A and 50% B for 2 min. The eluent composition was returned to 100% A and the column was equilibrated for 10 min prior to the next injection. Eluted carotenoid and chlorophyll compounds from a 20-μL injection were detected at 452, 652, and 665 nm and data was collected, recorded, and integrated using 1100 HPLC ChemStation Software (Agilent Technologies). Peak assignment was performed by comparing retention times and line spectra obtained from photodiode array detection with

authentic standards purchased from commercial vendors. Lutein and zeaxanthin are usually reported together in food composition tables because of their structural similarity.^[18]

Statistical Analysis

Data were analyzed by the GLM procedure of SAS (Version 8.2; Cary, NC) to perform analysis of variance, regression analysis, and orthogonal contrasts to determine relationships between dependent variables and N treatments.

RESULTS AND DISCUSSION

Tissue Biomass Accumulation

Parsley shoot tissue fresh weight (STFW) responded significantly to N treatment ($P \leq 0.001$). The dry weight of the shoot tissue (STDW) also responded significantly to N treatment ($P \leq 0.001$). Shoot tissue fresh weight of ten uniform parsley plants per treatment replication ranged from 80.5 g under 6 mg N L⁻¹ to 565.9 g under 105 mg N L⁻¹. The STDW of these samples ranged from 13.1 g under 6 mg N L⁻¹ to 70.5 g under 105 mg N L⁻¹. As the concentration of N in the nutrient solution was increased, linear increases were observed in STFW [g STFW = -55.3 + 123.3 trt, $r^2 = 0.98$, $P \leq 0.001$] and STDW [g STDW = 1.2 + 14.8 trt, $r^2 = 0.96$, $P \leq 0.001$]. These trends follow yield responses reported for other vegetable crops under increasing N fertility.^[19] However, Pasikowska et al.^[20] reported no significant effect of N at 50 to 120 kg ha⁻¹ on the yield of 'Festival' and 'Paramount' parsley on three different soil types.

Macro- and Micronutrient Accumulation

Macronutrient accumulation in the leaves of parsley responded significantly to N treatment concentrations. Significant responses to N treatments were observed for N ($P \leq 0.001$), P ($P \leq 0.001$), K ($P = 0.003$), Ca ($P \leq 0.001$), Mg ($P \leq 0.001$), and sulfur (S) ($P = 0.012$) in parsley leaf tissues. Trend analysis was determined by regression models. Percent N, P, and K in leaf tissue increased linearly with increasing N concentrations in the nutrient solution (Table 1). In a quadratic trend, Percent Ca, Mg, and S in leaf tissue first decreased, then increased in response to increasing N treatment concentrations (Table 1). Accumulation of macronutrient elements in response to increasing N treatment concentrations may have dietary nutritional importance. Parsley ranks behind only turnip greens (*Brassica rapa* L.), garlic (*Allium sativum* L.), and kale (*Brassica oleracea* var. *acephala* DC.) for Ca content, and behind beet greens (*Beta vulgaris* L.), potatoes (*Solanum tuberosum* L.), and spinach (*Spinacia oleracea* L.) for K content.^[5] The highest levels of tissue N, P, K, Ca, and S were found with 105 mg N L⁻¹, indicating that increases in N nutrition greatly enhance the macronutrient content of parsley leaves.

Parsley leaf micronutrient accumulation also responded significantly to N treatment concentrations (Table 2). Significant responses to N treatments were observed for B ($P \leq 0.001$), Cu ($P = 0.004$), Fe ($P = 0.009$), Mn ($P \leq 0.001$), Mo ($P \leq 0.001$), and Zn ($P = 0.066$). Leaf tissue B, Cu, and Zn first decreased, then increased as the concentration of N in the nutrient solutions increased from 6 to 105 mg N L⁻¹. Leaf tissue Fe, Mn, and Mo decreased linearly in response to increasing N treatment concentrations. Parsley ranks very high for Fe concentration per 100 g edible portion.^[5] Using a rat (*Rattus norvegicus* Berkenhout) bioassay, Welch et al.^[21] demonstrated that seed Fe content in bean (*Phaseolus vulgaris* L.) genotypes directly influenced intestinal absorption of Fe. Results from the current study demonstrate significant decreases in Fe concentration in the leaves of parsley with increasing levels of N in solution. Increasing N in the growing media would lower the expected concentration of Fe in parsley leaf tissues and, thus, decrease the contribution of Fe from parsley in the diet.

Carotenoid and Chlorophyll Accumulation

Lutein-zeaxanthin concentrations in the parsley shoot tissues responded significantly to N treatment concentrations ($P \leq 0.001$). The highest mean lutein-zeaxanthin concentration was under the highest N treatment concentration (Table 3). Previously, parsley had been reported to accumulate 10.2 to 13.8 mg lutein-zeaxanthin 100g⁻¹ FW.^[22,23] However, parsley values as low as 0.1 mg lutein-zeaxanthin 100 g⁻¹ FW are in the literature.^[24] Although the measured lutein-zeaxanthin concentration in this study is lower than the value listed by the USDA-NCI carotenoid database^[3], the parsley carotenoid concentration at the 105 mg N L⁻¹ treatment exceeds most cultivated vegetable crops. There was a linear increase in lutein-zeaxanthin as the concentration of N in the nutrient solutions increased [Lutein-zeaxanthin = 2.8 + 1.1 trt, $r^2 = 0.90$, $P \leq 0.001$]. β -carotene concentrations also responded significantly to N concentration ($P \leq 0.001$). The highest concentration of β -carotene was also with the highest N treatment (Table 3). β -carotene concentration in parsley shoots was within the previously published range of 2.3 to 8.0 mg 100g⁻¹ FW.^[22,23,24] Again, a linear increase occurred as the level of N in the nutrient solution increased [β -carotene = 2.7 + 1.0 trt, $r^2 = 0.90$, $P \leq 0.001$]. Results from the current study demonstrate the potential for carotenoid enhancement in parsley leaf tissues under increasing N fertility levels.

Chlorophyll concentrations dominated the pigment profile of the parsley leaves. Chlorophyll *a* (Chl *a*) responded significantly to N concentration treatment ($P \leq 0.001$), as did chlorophyll *b* ($P \leq 0.001$) (Table 3). The concentration of Chl *a* increased linearly as the N in solution was increased [Chl *a* = 44.8 + 21.8 trt, $r^2 = 0.88$, $P \leq 0.001$]. Chlorophyll *b* (Chl *b*) concentrations also increased with increasing N treatment concentrations [Chl *b* = 0.9 + 5.2 trt, $r^2 = 0.89$, $P \leq 0.001$]. Differences in pigmentation among the N treatments were visually apparent on growing plants before harvest and analysis. Parsley leaves at the lower N treatments tended to have a yellow hue, whereas the leaves at the higher N treatments took on a dark-green color.

Increased ingestion of vegetables high in lutein carotenoids has been associated with decreased risk of eye disease.^[25] Studies also indicate that consumption of vegetables providing a mixture of carotenoids were more associated with reducing cancer and eye disease risk than consumption of individual carotenoid supplements.^[26,27] Carotenoids serve antioxidant functions when consumed in the diet. In fact, carotenoids are the most potent biological quenchers of reactive oxygen species.^[28] Several reactive oxygen species (ROS) such as $^1\text{O}_2$, OH^- , O_2^- , and H_2O_2 are produced naturally in the human body during normal metabolism.^[29] In the human body, oxidants produced during normal metabolism and immune defense against infectious and chemical agents are responsible for damage to DNA, proteins, and cellular tissues.^[13,30] This harmful oxidative damage is considered the major cause of aging and degenerative diseases such as cancer, cardiovascular disease, immune-system decline, and cataract.^[30] Therefore, identifying ways to increase the overall dietary intake of carotenoids may help improve human health.

CONCLUSIONS

Parsley leaf biomass, elemental concentrations, and carotenoid pigments all displayed significant responses to increasing N concentrations from 6 mg N L^{-1} to 105 mg N L^{-1} in nutrient solution culture. Plant biomass increased with increasing N treatments. Concentrations of B, Ca, Cu, K, Mo, N, P, and S in parsley leaves increased with increasing N nutrition. Increasing N concentrations in the nutrient solutions significantly increased the concentrations of lutein-zeaxanthin and β -carotene carotenoids. Parsley ranks third on the USDA Nutrient Database for lutein-zeaxanthin and β -carotene content, and this study demonstrates that these carotenoids can be influenced by changes in N fertility levels. Increasing the carotenoid concentration in parsley would be expected to enhance the nutritional contribution of this culinary herbal crop.

ACKNOWLEDGEMENTS

This project was funded in part by a undergraduate student research award received by the Undergraduate Research Opportunities Program (UROP) at the University of New Hampshire. The manuscript partially fulfills the requirement for an honors senior thesis. This is Scientific Contribution Number ____ from the New Hampshire Agricultural Experiment Station.

REFERENCES

1. Simon, J.E.; Quinn, J. Characterization of essential oil of parsley. *J. Agric. Food Chem.* **1988**, *36*, 467-472.
2. López, M.G.; Sánchez-Mendoza, I.R.; Ochoa-Alejo, N. Comparative Study of Volatile Components and fatty acids of plants and in vitro cultures of parsley (*Petroselinum crispum* (Mill) Nym ex Hill). *J. Agric. Food Chem.* **1999**, *47*, 3292-3296.

3. Mangels, A.R.; Holden, H.M.; Beecher, G.R.; Forman, M.R.; Lanza, E. Carotenoid content of fruits and vegetables: An evaluation of analytic data. *J. Amer. Dietetic Assoc.* **1993**, *93* (3), 284-295.
4. Pennington, J.A.T.; Church, H.N. *Food Values of Portions Commonly Used*. 14th Ed.; J.B. Lippincott: Philadelphia, PA, 1985.
5. Maynard, D.N.; Hochmuth, G.J. *Knott's Handbook for Vegetable Growers*. 4th Ed.; John Wiley and Sons, Inc.: New York, NY, 1997.
6. Hochmuth, G.J. Nitrogen management practices for vegetables in Florida. *Fla. Agric. Exp. Sta. Cir. No. 1222*; Gainesville, FL, **2000**.
7. Mozafar, A. Nitrogen fertilizers and the amount of vitamins in plants: A review. *J. Plant Nutr.* **1993**, *16* (12), 2479-2506.
8. Hochmuth, G.J.; Brecht, J.K.; Bassett, M.J. Nitrogen fertilization to maximize carrot yield and quality on a sandy soil. *HortScience* **1999**, *34* (4), 641-645.
9. Zaripheh, S.; Erdman, Jr., J.W. Factors that influence the bioavailability of xanthophylls. *J. Nutr.* **2002**, *132*, 531S-534S.
10. Frank, H.A.; Cogdell, R.J. Carotenoids in photosynthesis. *Photochemistry* **1996**, *63* (3), 257-264.
11. Havaux, M.; Niyogi, K.K. The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proc. Natl. Acad. Sci.* **1999**, *96*, 8762-8767.
12. Sommerburg, O.; Siems, W.G.; Hurst, J.S.; Lewis, J.W.; Kliger, D.S.; Kuijk, F.J. Lutein and zeaxanthin are associated with photoreceptors in the human retina. *Current Eye Res.* **1999**, *19* (6), 491-495.
13. Mortensen, A.; Skibsted, L.H.; Truscott, T.G. The interaction of dietary carotenoids with radical species. *Arch. Biochem. Biophys.* **2001**, *385* (1), 13-19.
14. Hoagland, D.R.; Arnon, D.I. The water culture method for growing plants without soil. *Calif. Agric. Exp. Sta. Circ. No. 347*; Berkeley, CA, **1950**.
15. Eidsten, I.M.; Gislerød, H.R. Growing of curled parsley in nutrient film. *Acta Hort.* **1986**, *178*, 223-226.
16. Focht, C.L., ed. *Plants*. In: W.H. Horwitz, ed. *Official Methods of Analysis of AOAC International*. 17th ed. Agricultural chemicals; contaminants; drugs, vol. 1. Gaithersburg, MD: AOAC International, 2000, pp. 3.1-3.37.
17. Khachik, F.; Beecher, G.R.; Whittaker, N.F. Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. Agr. Food Chem.* **1986**, *34* (4), 603-616.
18. Holden, J.M.; Eldridge, A.L.; Beecher, G.R.; Buzzard, I.M.; Bhagwat, S.; Davis, C.S.; Douglass, L.W.; Gebhardt, S.; Haytowitz, D.; Schakel, S. Carotenoid content of U.S. foods: An update of the database. *J. Food Comp. Anal.* **1999**, *12*, 169-196.
19. Greenwood, D.J.; Cleaver, T.J.; Turner, M.K.; Hunt, J.; Niendorf, K.B.; Loquens, S.M.H. Comparison of the effects of nitrogen fertilizer on the yield, nitrogen content and quality of 21 different vegetable and agricultural crops. *J. Agric. Sci. Camb.* **1980**, *95*, 471-485.
20. Pasikowska, R.; Dabrowska, B.; Capecka, E. The effect of nitrogen fertilization rate on the yield and quality of two cultivars of parsley (*Petroselinum sativum* L. ssp. *crispum*) grown on different soil types. *Folia Horticulturae* **2002**, *14* (1), 177-185.

21. Welch, R.M.; House, W.A.; Beebe, S.; Senadhira, D.; Gregorio, G.; Cheng, Z. Testing iron and zinc bioavailability in genetically enriched bean (*Phaseolus vulgaris* L.) and rice (*Oryza sativa* L.) using a rat model. *Food Nutr. Bul.* **2000**, *21*, 428-433.
22. Müller, H. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z Lebensm Unters Forsch A* **1997**, *204*, 88-94.
23. Heinonen, M.I.; Ollilainen, V.; Linkola, E.K.; Varo, P.T.; Koivistoinen, P.E. Carotenoids in finnish foods: Vegetables, fruits, and berries. *J. Agric. Food Chem.* **1989**, *37*, 655-659.
24. Ben-Amotz, A.; Fishler, R. Analysis of carotenoids with emphasis on 9-*cis* **b**-carotene in vegetables and fruits commonly consumed in Israel. *Food Chem.* **1998**, *62* (4), 515-520.
25. Jaques, P.F.; Chylack, L.T. Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. *Amer. J. Clin. Nutr.* **1991**, *53*, 352S-355S.
26. Johnson, E.J.; Hammond, B.R.; Yeum, K.J.; Qin, J.; Wang, X.D.; Castaneda, C.; Snodderly, D.M.; Russell, R.M. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Amer. J. Clinical Nutr.* **2000**, *71*, 1555-1562.
27. Le Marchand, L.; Hankin, J.H.; Kolonel, L.N.; Beecher, G.R.; Wilkens, L.R.; Zhao, L.P. Intake of specific carotenoids and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.* **1993**, *2*, 183-187.
28. DiMascio, P.; Kaiser, S.; Sies, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* **1989**, *274*, 532-538.
29. Young, A.J.; Lowe, G.M. Antioxidant and Prooxidant Properties of Carotenoids. *Arch. Biochem. Biophys.* **2001**, *385* (1), 20-27.
30. Ames, B.N.; Shigenage, M.K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.* **1993**, *90*, 7915-7922.

Table 1. Mean values of macroelemental accumulation in the leaf tissues of ‘Dark Green Italian’ parsley grown under increasing nitrogen concentrations in nutrient solution culture.

Treatment mg N L ⁻¹	Elemental composition ^a (%)					
	N	P	K	Ca	Mg	S
6	1.83 ± 0.17	0.72 ± 0.03	3.75 ± 0.30	1.03 ± 0.13	0.41 ± 0.04	0.27 ± 0.02
13	1.83 ± 0.15	0.68 ± 0.09	3.91 ± 0.40	0.94 ± 0.05	0.25 ± 0.03	0.23 ± 0.04
26	2.20 ± 0.08	0.70 ± 0.08	3.83 ± 0.22	0.86 ± 0.11	0.24 ± 0.03	0.24 ± 0.04
52.5	3.30 ± 0.11	0.79 ± 0.06	4.44 ± 0.25	0.71 ± 0.03	0.27 ± 0.01	0.30 ± 0.01
105.0	4.75 ± 0.10	0.92 ± 0.02	4.49 ± 0.49	1.16 ± 0.12	0.37 ± 0.04	0.33 ± 0.06
Contrasts ^b						
Linear	***	**	**	NS	NS	*
Quadratic	***	**	**	**	***	***

^a Mean composition of sampled leaf tissue of 4 replications, 10 plants each, ± standard error.

^b NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01, or 0.001 level, respectively.

Table 2. Mean values of microelemental accumulation in the leaf tissues of ‘Dark Green Italian’ parsley grown under increasing nitrogen concentrations in nutrient solution culture.

Treatment mg N L ⁻¹	Elemental composition ^a (mg kg ⁻¹)					
	B	Cu	Fe	Mn	Mo	Zn
6	31.07 ± 1.26	5.33 ± 0.50	263.67 ± 48.25	192.00 ± 16.96	6.43 ± 1.10	47.67 ± 16.56
13	25.87 ± 3.31	5.13 ± 1.03	166.67 ± 52.61	105.00 ± 18.23	2.54 ± 0.50	31.00 ± 13.66
26	30.43 ± 0.97	5.70 ± 0.76	161.33 ± 37.49	85.34 ± 13.02	1.64 ± 0.80	26.00 ± 3.36
52	33.67 ± 3.15	6.34 ± 0.52	155.33 ± 21.60	71.00 ± 3.15	0.47 ± 0.38	30.33 ± 13.66
105	37.57 ± 1.87	6.77 ± 0.79	145.34 ± 44.46	86.33 ± 12.14	0.97 ± 0.50	31.67 ± 9.26
Contrasts ^b						
Linear	***	**	**	***	***	NS
Quadratic	***	**	**	***	***	*

^a Mean composition of sampled leaf tissue of 4 replications, 10 plants each, ± standard error.

^b NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01, or 0.001 level, respectively.

Table 3. Mean pigment content in mg 100 g⁻¹ FW of leaf tissues of ‘Dark Green Italian’ parsley grown under increasing nitrogen concentrations in nutrient solution culture.

Treatment mg N L ⁻¹	Pigment composition ^a (mg 100 g ⁻¹ FW)			
	Lutein- zeaxanthin	β-Carotene	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>
6	4.30 ± 0.36	3.97 ± 0.41	75.89 ± 7.80	16.37 ± 1.78
13	4.71 ± 0.30	4.60 ± 0.15	79.92 ± 6.81	17.52 ± 1.33
26	5.99 ± 0.22	5.41 ± 0.38	104.66 ± 5.94	22.98 ± 0.63
52	7.34 ± 0.57	6.97 ± 0.77	131.02 ± 13.52	29.73 ± 2.13
105	8.56 ± 0.83	7.85 ± 0.45	159.19 ± 16.62	36.14 ± 3.99
Contrasts ^b				
Linear	***	***	***	***

^a Mean composition of sampled leaf tissue of 4 replications, 10 plants each, ± standard error.

^b *** significant at $P \leq 0.001$.