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Effect of Organic and Conventional Cropping Systems on Ascorbic Acid, Vitamin C, Flavonoids, Nitrate, and Oxalate in 27 Varieties of Spinach (*Spinacia oleracea* L.)

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ABSTRACT: This study was undertaken to compare the levels of ascorbic acid, vitamin C, flavonoids, nitrate, and oxalate in 27 spinach varieties grown in certified organic and conventional cropping systems. Liquid chromatography−electrospray ionization−tandem mass spectrometry (LC−(ESI)MS/MS) of methanolic extracts of spinach demonstrated 17 flavonoids, including glucuronides and acylated di- and triglycosides of methylated and methylenedioxyderivatives of 6-oxygenated flavonoids. The mean levels of ascorbic acid and flavonoids were significantly ($p < 0.001$) higher in the organically grown [40.48 ± 6.16 and 2.83 ± 0.03 mg/kg of fresh weight (FW)] spinach compared to the conventionally grown spinach (25.75 ± 6.12 and 2.27 ± 0.02 mg/kg of FW). Conversely, the mean levels of nitrate were significantly ($p < 0.001$) higher in the conventionally grown spinach compared to the organically grown spinach. No significant effects were observed in the oxalate content of spinach from either production system. The levels of nitrate correlated negatively with those of ascorbic acid, vitamin C, and total flavonoids and showed a positive correlation with the oxalate content. These results suggest that organic cropping systems result in spinach with lower levels of nitrates and higher levels of flavonoids and ascorbic acid.

KEYWORDS: *Spinach, organic, conventional, ascorbic acid, vitamin C, flavonoids, nitrate, oxalate, HPLC*

INTRODUCTION

Spinach (*Spinacia oleracea* L.) is a leafy vegetable commonly consumed fresh or as canned or frozen products. In the United States, the consumption of fresh spinach per capita averaged 1.9 lb (in 2009), which accounts for about three-fourths of all spinach consumed.[1] Spinach is a good source of vitamin C [28.1 mg/100 g of fresh weight (FW)], vitamin A (9377 IU/100 g of FW), carotenoids (17.8 mg/100 g of FW), flavonoids (5.99 mg/100 g of FW), folic acid (194 μg/100 g of FW), and minerals, such as calcium and magnesium.[2,3] Spinach can also contain high levels of nitrate (average of 217 mg/100 g of FW in U.S. spinach) and oxalate (400−900 mg/100 g of FW), which can be detrimental to human health.[4,5] About 5% of consumed nitrate is reduced to nitrite in the gastrointestinal tract. Upon absorption, nitrite is oxidized to nitrate via a reaction with hemoglobin, converting it to methemoglobin, which can no longer bind oxygen and may result in methemoglobinemia, especially in infants and small children.[6,7] Absorbed nitrate can also form carcinogenic nitrosamines.[7,8] Oxalate acts as an antinutrient by binding minerals, such as calcium and magnesium, resulting in lower bioavailability.[9] Excessive intake of oxalate may lead to the formation of renal calcium stones.[10] For these reasons, the Scientific Committee for Food (SCF) established an acceptable daily intake (ADI) for nitrate as 3.7 mg/kg of body weight, and the European Commission set maximum limits of nitrogen accumulation in spinach as 3000 and 2500 ppm FW for winter and spring crops, respectively.[11] Increasingly, objectives of contemporary spinach breeding programs include identifying cultivars low in nitrate and oxalate as well as high in bioactive compounds with health-promoting activity, such as flavonoids and ascorbic acid.

Reviews of studies comparing the nutrient content of conventional and organic crops have demonstrated inconsistent differences, with the exception of higher levels of ascorbic acid and less nitrate in organic products.[12−14] However, these data are difficult to interpret because cultivar selection/identification, sampling, analytical methods, and agronomic conditions varied in the literature cited. In a recent review of the more rigorously controlled comparison studies during 2000−2006, Mitchell et al. noted a similar trend of higher levels of ascorbic acid (vitamin C) and phenolic compounds and lower levels of nitrate in organic tomatoes compared to their conventional counterparts.[15] To understand these relationships, more studies encompassing a wider variety of crops are still needed because various species and cultivars do not respond to agronomic and environmental pressures equivocally.

Accordingly, the aims of this study were to (1) determine the levels of ascorbic acid, vitamin C, flavonoids, nitrate, and oxalate in 27 different spinach cultivars, (2) evaluate the influence of certified organic and conventional cultivation methods on these components, and (3) identify varietal/cultivation combinations that may lead to an improvement in the quality of spinach.

MATERIALS AND METHODS

Chemicals. Spiraeoside (2-hydroxy-4-[3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl]phenyl *β*-D-glucopyranoside) was obtained from Extrasynthese (Lyon, France). Tetrabutyl ammonium chloride was

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Table 1. Content of Ascorbic Acid, Vitamin C, and Total Flavonoids in 27 Varieties of Spinach Grown under Conventional and Organic Agricultural Conditions[a]

	ascorbic acid (mg/100 g of FW)		vitamin C[b] (mg/100 g of FW)		total flavonoids (mg/100 g of FW)	
variety	conventional	organic	conventional	organic	conventional	organic
501	34.38 ± 12.11	45.72 ± 10.30	42.95 ± 7.13	53.52 ± 8.20	2.70 ± 0.10	3.09 ± 0.22
507	29.36 ± 7.85	45.94 ± 14.78	41.86 ± 8.31	54.16 ± 9.68	2.21 ± 0.32	2.64 ± 0.45
509	29.46 ± 9.59	43.85 ± 14.71	43.56 ± 16.61	52.34 ± 13.50	2.37 ± 0.09	2.85 ± 0.48
510	20.04 ± 4.44	44.50 ± 8.83	30.21 ± 6.02	55.08 ± 10.87	2.84 ± 0.26	3.11 ± 1.14
515	36.66 ± 28.19	39.14 ± 15.21	44.98 ± 22.49	47.72 ± 13.13	2.17 ± 0.22	3.05 ± 1.15
616	19.36 ± 13.49	32.40 ± 9.19	31.18 ± 15.10	41.04 ± 8.24	2.29 ± 0.59	2.95 ± 0.05
617	21.98 ± 7.79	35.83 ± 6.75	35.44 ± 1.85	43.14 ± 8.38	2.12 ± 0.25	2.98 ± 0.24
618	40.45 ± 17.63	39.95 ± 13.38	48.49 ± 12.96	49.32 ± 10.78	2.41 ± 0.48	3.31 ± 0.56
619	16.42 ± 3.69	41.39 ± 12.24	26.41 ± 4.31	51.89 ± 13.90	2.23 ± 0.21	2.85 ± 0.66
620	19.01 ± 4.17	27.33 ± 19.29	29.37 ± 5.08	35.98 ± 14.91	1.45 ± 0.15	1.62 ± 0.45
621	24.44 ± 6.17	50.02 ± 19.48	33.43 ± 5.22	58.91 ± 18.22	2.27 ± 0.17	2.92 ± 0.92
622	34.52 ± 24.43	41.78 ± 5.89	44.43 ± 23.01	50.74 ± 8.17	1.74 ± 0.19	2.32 ± 0.65
623	24.96 ± 19.96	29.94 ± 11.56	37.59 ± 17.82	36.91 ± 13.21	2.27 ± 0.40	2.54 ± 0.86
Winter Bloomsdale	26.46 ± 6.66	33.03 ± 7.33	37.93 ± 6.26	45.88 ± 8.91	2.09 ± 0.21	2.70 ± 0.06
Winter Giant	17.34 ± 2.69	30.88 ± 8.63	27.32 ± 3.62	37.53 ± 7.01	2.30 ± 0.22	2.57 ± 0.33
Viroflay	22.00 ± 8.55	41.48 ± 5.08	30.41 ± 11.56	49.17 ± 4.33	2.48 ± 0.34	3.01 ± 0.33
America	19.13 ± 6.38	46.74 ± 24.86	28.30 ± 6.94	53.12 ± 22.52	2.24 ± 0.33	3.05 ± 0.52
LS Bloomsdale	24.34 ± 10.97	43.33 ± 32.68	32.41 ± 9.94	49.81 ± 29.07	2.49 ± 0.18	2.94 ± 0.64
Space	36.80 ± 20.50	49.06 ± 10.47	42.18 ± 17.49	55.01 ± 9.24	2.14 ± 0.29	2.75 ± 0.65
Whale	33.29 ± 23.96	40.37 ± 16.26	41.49 ± 21.62	48.84 ± 18.79	2.32 ± 0.81	2.69 ± 0.34
Spargo	37.19 ± 19.06	48.46 ± 25.44	46.49 ± 13.66	56.05 ± 23.41	2.71 ± 0.33	3.16 ± 0.86
Tyee	25.23 ± 2.81	53.71 ± 21.72	34.98 ± 1.81	62.87 ± 16.61	2.83 ± 0.52	4.47 ± 0.40
Tarpy	23.94 ± 1.78	27.36 ± 1.35	31.61 ± 2.18	34.17 ± 1.49	2.59 ± 0.10	2.55 ± 0.48
Samish	13.48 ± 10.55	27.23 ± 4.21	24.65 ± 5.62	35.61 ± 5.05	1.95 ± 0.38	2.55 ± 0.39
Renegade	21.46 ± 8.89	51.59 ± 21.00	29.40 ± 8.22	58.71 ± 18.53	2.01 ± 0.19	2.71 ± 0.08
Spalding	16.15 ± 9.19	39.15 ± 23.74	24.57 ± 9.69	46.72 ± 23.99	2.01 ± 0.74	2.30 ± 0.87
Pelican	27.26 ± 5.58	42.75 ± 21.89	35.02 ± 4.82	48.21 ± 19.26	1.97 ± 0.32	2.61 ± 0.23
average	25.75 ± 6.12	40.48 ± 6.16	35.43 ± 6.09	48.61 ± 6.06	2.27 ± 0.023	2.83 ± 0.031

[a]Values are reported as the mean ± standard deviation. [b]Vitamin C value was based on the sum of ascorbic acid and dehydroacsorbic acid.

purchased from Fluka (Buchs, Switzerland). Potassium nitrate, potassium nitrite, potassium phosphate monobasic, potassium phosphate dibasic, ascorbic acid, *m*-phosphoric acid (85%), and high-performance liquid chromatography (HPLC) solvents were from Fisher Scientific (Fair Lawn, NJ). D,L-1,4-Dithiothreital (DTT) was purchased from Acros Organics (Geel, Belgium).

A total of 27 spinach varieties were grown at a certified organic farm and a conventional farm in the northwestern United States. The farms were located 34 km apart from each other. Farms were selected to obtain the comparative pairs within the same environmental conditions: conventional (Whidbey Island, WA; latitude, 48° 24′ N; longitude, 122° 65′ W; elevation, 53.34 m) and certified organic (Port Townsend, WA; latitude, 48° 12′ N; longitude, 122° 81′ W; elevation, 30.48 m). During spinach cultivation, the temperatures of both farms were similar, with the range of 4.8−24.4 °C in the organic farm and 0−20 °C in the conventional farm. The organically managed spinach field was in certified organic cultivation for 10 years with lettuce planting prior to this spinach rotation. The conventional field was planted with spinach/fallow in the same year and had been under the conventional practice for the past 10 years. The soil was classified as sandy loam in both fields. Plots in both systems were arranged in a randomized complete block design with three replications. The plot size was 2.7 m^2 (3.0 × 0.9 m), with three rows per bed. All varieties of spinach were sown on August 28, 2007 and harvested on November 1, 2007. The conventionally managed field received pre-plant-incorporated synthetic fertilizer (20-10-10 NPK). The organic field was fertilized with pre-plant-incorporated compost. The application rate was 2553 ft^3/acre. The compost consisted of vegetative wastes, horse manure, straw bedding, and salmon wastes, of 0.5 in. thick across beds. Plants were irrigated using overhead sprinklers. Weed control at the organic site was achieved by hand hoeing and thinning. Weed control in the conventional site was achieved by mechanical tractor-mounted cultivation. Pesticides were not used in either cropping system because no significant insect damage incurred.

Spinach Sampling. Nine plants per plot were randomly selected, and five leaves per plant were picked from the middle stage of growth to avoid the newest and oldest leaves. A total of 45 leaves per plot were then bagged and immediately packed in a cardboard box lined with styrofoam. The box wrapped in plastic was kept under cool temperature using bags of dry ice to retain susceptible compounds to high temperature and immediately shipped to the University of California at Davis through overnight delivery. Upon arrival, spinach was washed, dried, packaged in a vacuum plastic bag, and frozen using a blasting freezer. Spinach samples were stored at −80 °C until analyses could be performed.

Analysis of Ascorbic Acid. The ascorbic acid determination was based on the method by Sánchez-Mata et al., with a slight modification.[16] Approximately 10 g of pulverized frozen spinach samples was mixed with 25 mL of 2.5% *m*-phosphoric acid on a vortex mixer for 1 min and centrifuged at 3000*g* for 20 min at 4 °C. The supernatant was filtered through a cheese cloth into a 50 mL volumetric flask, and the residue was re-extracted with 20 mL of 2.5% *m*-phosphoric acid, centrifuged, filtered into the same volumetric flask, brought to volume, and filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane with a glass microfiber prefilter (Whatman, Florham Park, NJ). This extract was used to measure ascorbic acid. For vitamin C determination, 1.00 mL of the filtrate was treated with 0.20 mL of DTT (40 mg/mL) at 40 °C for 2 h for the reduction of dehydroascorbic acid into ascorbic acid. Extracts were analyzed using a Hewlett-Packard series 1090 liquid chromatograph (Agilent, Palo Alto, CA) equipped with a diode array detector monitoring at 245 nm. Separations were achieved on an Agilent Zorbax XDB C_{18} column (4.6 × 250 mm, 5 μm) fitted with a guard column (4.6 × 12.5 mm, 5 μm) of the same material (Santa Rosa, CA). An isocratic separation was performed using 0.05 M KH_2PO_4 (pH 2.6) at 1.0 mL/min. The linear range of quantification of ascorbic acid was 0.005−0.200 mg/mL.

Analysis of Flavonoids. The flavonoid determination was based on the method by Gil et al.[17] About 10.0 g of fresh spinach was homogenized with 50 mL of the mixture of methanol/water (5:95, v/v) containing citric acid (0.5 g/L) with ethylenediaminetetraacetic acid (EDTA) (0.5 g/L) for 1 min. The homogenate was filtered through a cheese cloth into a 50 mL volumetric flask, brought to volume, and filtered through a 0.45 μm filter. Extracts were analyzed using a Hewlett-Packard series 1090 liquid chromatograph (Agilent, Palo Alto, CA) equipped with a diode array detector. Separations were achieved on an Agilent Zorbax XDB C_{18} column (4.6 × 250 mm, 5 μm) with a guard column (4.6 × 12.5 mm, 5 μm) of the same material (Santa Rosa, CA). The mobile phase consisted of formic acid/water (19:1, v/v) (A) and methanol (B). The elution was performed on a gradient starting with 10% B in A to reach 40% at 30 min and 80% B at 40 min at a flow rate of 1 mL/min. Flavonoids were quantified at 350 nm using an external spiraeoside standard (Extrasynthese, Lyon, France) because authentic standards were not available. The linear range of quantification of spiraeoside was 0.0001−0.2500 mg/mL. Structural identification of peaks with molecular ions corresponding to flavonoids was achieved by product ion scanning of liquid chromatography−electrospray ionization−tandem mass spectrometry (LC−(ESI)MS/MS, Micromass, Altrincham, U.K.). The triple quadrupole ESI source parameters were the following: capillary, 3.0 kV in the negative mode; extractor, 2 V; source block, 145 °C; desolvation temperature, 340 °C; nitrogen gas, 460 L/h; and cone gas, 67 L/h. The total ion chromatogram (TIC) was monitored at a mass range of m/z 100−1200. The cone voltages varied from 13 to 45 V to optimize the intensity of the product ions.

Analysis of Nitrate and Oxalate. The determination of nitrate and oxalate was based on the method by Kaminishi and Kita.[18] Approximately 10 g of frozen spinach was homogenized with 100 mL of deionized water. The mixture was extracted at room temperature for 10 min using a Lab-line Orbit Environ-Shaker (Lab-line Instruments, Inc., Melrose Park, IL). Homogenates were immediately filtered through filter paper (Whatman No. 41, Whatman International, Ltd., Maidstone, England). The filtrates were further filtered using a 0.45 μm PTFE membrane with a glass microfiber prefilter (Whatman, Florham Park, NJ). HPLC analysis was performed using the Agilent Technologies 1200 series (Waldbronn, Germany) monitoring at 210 nm. Isocratic separation was achieved using a Prodigy 5 μm ODS3 100A column (250 × 4.6 mm, 5 μm, Phenomenex, Torrance, CA) fitted with a guard column (4.6 × 12.5 mm, 5 μm) of the same material. The mobile phase consisted of 5 mM tetrabutyl ammonium chloride in phosphate buffer (pH 8.4) at 1.5 mL/min. The linear range of quantification for nitrate and oxalate was determined as 0.1−1.0 and 1.0−5.0 mg/mL, respectively.

Statistical Analysis. Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 16.0, SPSS, Inc., Chicago, IL). Data were subjected to analysis of variance using a general linear model to determine the influence of cropping systems and spinach genotype. Associations among micronutrient and nitrate levels in spinach were determined using Pearson's correlation analysis.

Table 2. Multivariate Analysis of Variance for the Cropping System and Spinach Cultivars[a]

	ascorbic acid	vitamin C	total flavonoids	nitrate	oxalate
cropping system (CS)	<0.001	<0.001	<0.001	<0.001	0.544
variety (V)	0.381	0.250	<0.001	<0.001	0.208
CS × V	0.981	0.906	0.961	0.165	0.055

[a]Units are expressed as p values for statistical significance.

RESULTS AND DISCUSSION

Ascorbic Acid and Vitamin C. Among 27 spinach cultivars studied, the levels of ascorbic acid and vitamin C (ascorbic acid and dehydroacsorbic acid) ranged from 13.48 to 53.71 mg/100 g of FW and from 24.57 to 62.87 mg/100 g of FW, respectively (Table 1). The mean vitamin C value reported herein for both the organic (48.61 ± 6.05 mg/100 g of FW) and conventional (35.43 ± 6.08 mg/100 g of FW) cropping systems are higher than the mean United States Department of Agriculture (USDA) reported value (28.1 ± 4.1 mg/100 g of FW) and lower than the result reported by Gil et al. (75 ± 5 mg/100 g of FW). This may result from various factors, including genotypes analyzed, postharvest handling, and the fact that this spinach was kept on ice immediately after harvest, slowing the degradation rate of vitamin C. The content of both ascorbic acid and vitamin C was significantly (p < 0.001) higher in the organically grown spinach compared to the conventionally grown spinach (Table 2). Genotype is known as the most influential factor determining the levels of ascorbic acid in vegetables.[19,20] Although it is difficult to make global statements relating levels of any individual bioactive compound with cultivation practices across the wider agronomic landscape, a tendency toward higher vitamin C levels in organically grown crops has now been reported in several crops.[13,15,21] This result supports the carbon/nitrogen balance theory of a increased growth and biomass in conventionally produced spinach

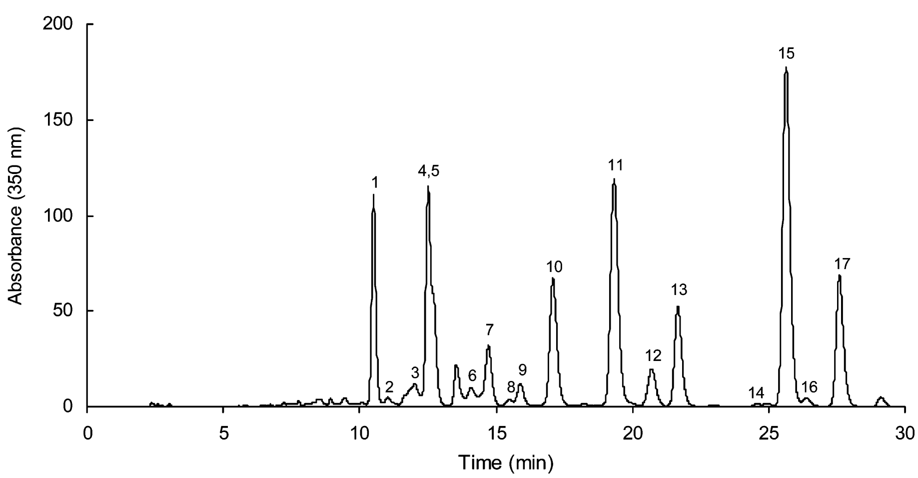
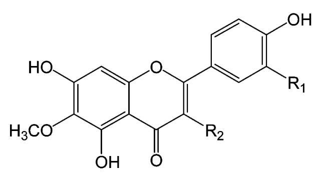


Figure 1. HPLC profile of 17 flavonoids identified in spinach monitoring 350 nm. Peak assignments are given in Table 3.

Table 3. HPLC t_R, Negative Ion m/z, and Important MS/MS Fragment Ions of 17 Flavonoids Detected in Spinach

number	RT	compound	$[M - H]^-$	fragments
1	10.32	patuletin-3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside	787	655, 331
2	11.50	patuletin-3-*O*-β-D-(2″-β-coumaroylglucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside	933	787, 331
3	11.91	patuletin-3-*O*-β-D-(2″-feruloylglucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside	963	787, 331
4	12.41	spinacetin-3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside	801	669, 345
5	12.41	patuletin-3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside	655	331
6	14.07	spinacetin-3-*O*-β-D-(2″-β-coumaroylglucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside	947	801, 507, 345
7	14.72	spinacetin-3-*O*-β-D-(2″-feruloylglucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside	977	801, 345
8	15.52	patuletin-3-*O*-β-D-(2″-coumaroylglucopyranosyl-(1→6)-β-D-glucopyranoside	801	655, 331
9	15.92	patuletin-3-*O*-β-D-(2″-feruloylglucopyranosyl-(1→6)-β-D-glucopyranoside	831	655, 331
10	17.15	spinacetin-3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside	669	345
11	19.40	spinatoside-4′-glucuronide	521	345
12	20.80	spinacetin-3-*O*-β-D-(2″-feruloylglucopyranosyl-(1→6)-β-D-glucopyranoside	845	669, 499, 345
13	21.75	jaceidin-4′-glucuronide	535	359
14	24.72	isomer of peak 12	845	669, 499, 345
15	25.74	5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-β-D-glucuronide	519	343
16	26.47	5,4′-dihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-β-D-glucuronide	503	327
17	27.68	5,4′-dihydroxy-3,3′-dimethoxy-6:7-methylenedioxyflavone-4′-β-D-glucuronide	533	357



Aglycone	R_1	R_2
Patuletin	OH	OH
Spinacetin	OCH_3	OH
Spinatoside	OH	OCH_3
Jaceidin	OCH_3	OCH_3

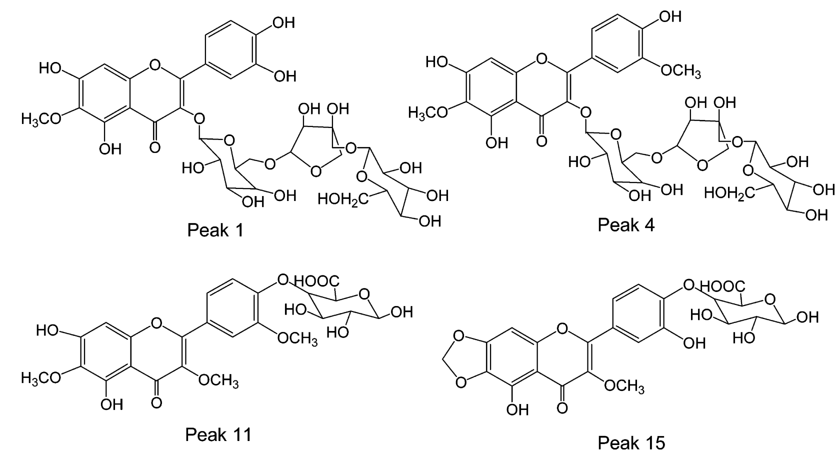


Figure 2. Chemical structure of flavonoid aglycones and major flavonoid glycosides identified in spinach. Structural identification of peaks with molecular ions corresponding to each flavonoid was achieved by product ion scanning of LC−ESI(−)−MS/MS.

because of nitrogen availability, which may have a diluting effect on the production of ascorbic acid. No significant difference was found between vitamin C and variety (Table 2). However, it is important to point out that multiple year studies using the same varieties would be needed to truly evaluate varietal differences.

Flavonoids. The analysis by LC−(ESI)MS/MS of methanolic extracts of spinach revealed the presence of 17 flavonoids, including glucuronides and acylated di- and triglycosides of methylated and methylenedioxyderivatives of 6-oxygenated flavonoids (Figure 1 and Table 3). Flavonoids found in spinach were the derivatives of patuletin (six peaks), spinacetin (five peaks), spinatoside (two peaks), jaceidin (one peak), and flavone (three peaks) (Figure 2 and Table 3). In addition, six flavonoids (peaks 11 and 13−17) in spinach were present as glucuronide conjugates. In most edible plants, flavonoids exist predominantly as glycosides of flavonoids. This is in agreement with earlier literature.[22,23] Two major flavonoids, spinatoside-4′-glucuronide (peak 11) and 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-β-D-glucuronide (peak 15) accounted for 12−19 and 19−26% of the total flavonoid content, respectively (Figure 3), which was consistent in all cultivars studied. Of the 17 flavonoids determined, the levels of 10 were higher in the organic spinach compared to the conventional spinach (Figure 3). In particular, glucuronide conjugates (peaks 13 and 15−17) were significantly ($p < 0.001$) higher in organic spinach compared to the conventional spinach. Such findings indicate that cultivation systems affected flavonoid synthesis, the degree of which depends upon the individual flavonoid.

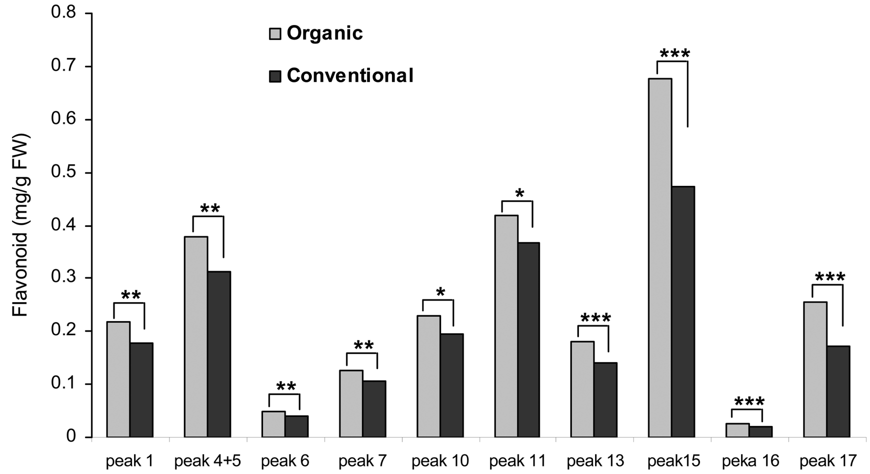


Figure 3. Comparison of the predominant flavonoid levels in organic and conventionally grown spinach. Flavonoid descriptions are given in Figure 1 and Table 3. *, **, and *** mean a significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, using Duncan's test.

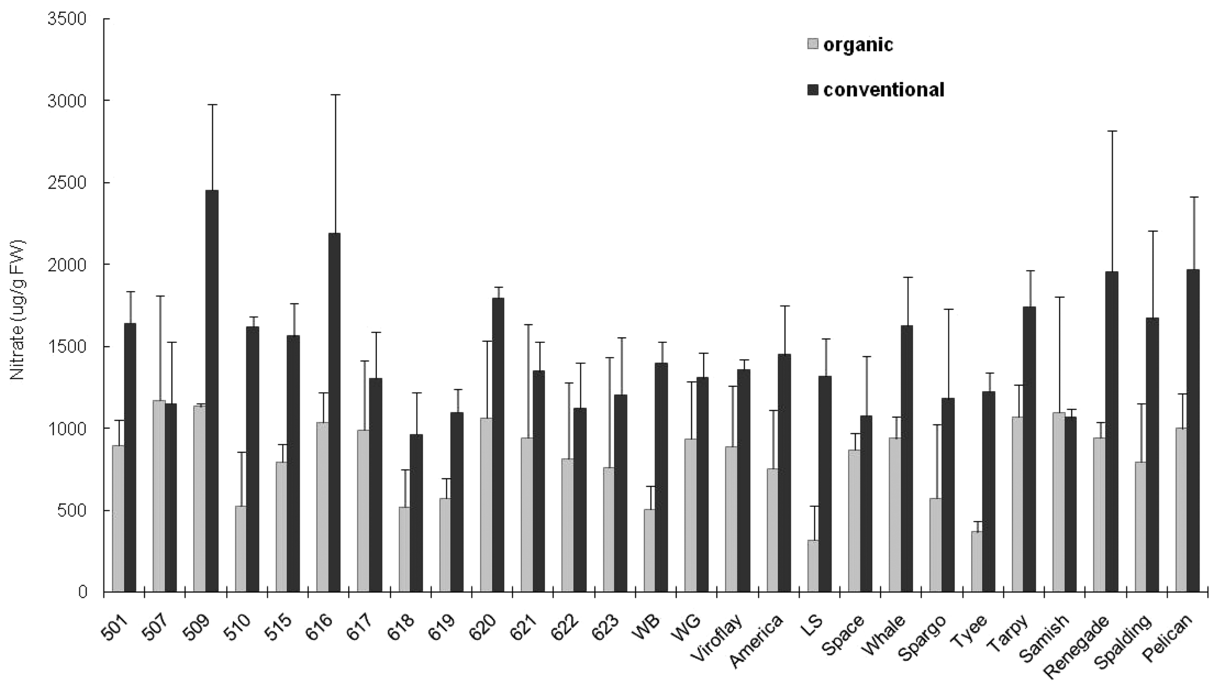


Figure 4. Nitrate levels in the 27 spinach cultivars grown in organic and conventional cropping systems. Values are expressed as the average of three composite values based on a FW basis.

The content of total flavonoids ranged from 1.92 to 4.82 mg/100 g of FW in organic spinach and from 1.45 to 2.84 mg/kg of FW in conventional spinach, respectively (Table 1). This is close to the value (1.8−3.7 mg/kg of FW) reported by Cho et al.[23] for spinach. The total flavonoid content and composition in spinach are influenced by genotype, growth stage, and postharvest handling.[17,24,25] Advanced breeding lines of spinach, with enhanced disease resistance, tend to have higher levels of individual and total flavonoids.[17,24] Herein, the levels of total flavonoids were significantly ($p < 0.001$) higher in the organic spinach (Table 2). Only one cultivar, Tarpy, was found to have a slightly higher mean value of total flavonoids in the conventional spinach (Table 1).

Nitrate and Oxalate. The nitrate content varied in organic (31.63−117.04 mg/100 g of FW) and conventionally grown spinach (96.13−245.35 mg/100 g of FW), and results are given in Figure 4. These values are similar to results (4.7−597.5 mg/g of FW) reported by Kaminishi and Kita[18] and Jaworska.[26] The levels of nitrate in spinach depend upon many factors, including variety,[27,28] fertilizer application,[29−31] and growing conditions.[28,32] Cantliffe reported significant differences in the nitrate content between smooth and savoyed leafed varieties of spinach. The nitrate concentration in spinach was found to increase significantly with nitrogen application rates.[29−31] Meteorological conditions, including air temperature, insolation length, and precipitation, were reported to make the greatest contribution (47%) to the nitrate content in potato and maize when compared to variety, fertilizers, soil fertility, and interaction of factors.[33] In this study, climate effects can be excluded because spinach was grown in the same area. This allowed for the evaluation of the cropping system and variety as factors determining the nitrate pool in spinach. Previous studies have demonstrated that the levels of nitrate are often higher in conventional produce compared to organic produce.[14,15,21]

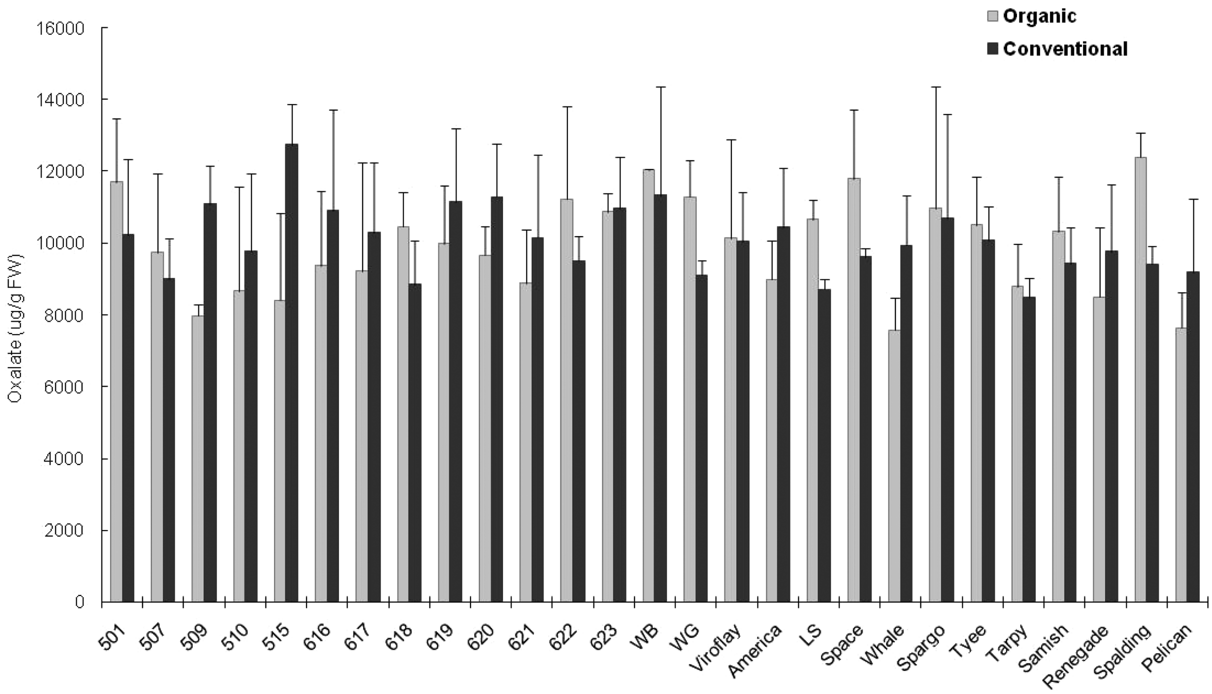


Figure 5. Oxalate levels in the 27 spinach cultivars grown in organic and conventional cropping systems. Values are expressed as the average of three composite values based on a FW basis.

Table 4. Pearson Correlation Coefficients between the Levels of Ascorbic Acid, Vitamin C, Total Flavonoids, Nitrate, and Oxalate in Spinach on the FW Basis

	vitamin C	total flavonoids	nitrate	oxalate
ascorbic acid	0.980[a]	0.404[a]	−0.370[a]	−0. 090
vitamin C		0.432[a]	−0.379[a]	−0.065
total flavonoids			−0.507[a]	−0.154
nitrate				0.155[b]

[a]A significant difference at $p < 0.01$ using Duncan's test. [b]A significant difference at $p < 0.05$ using Duncan's test.

The content of nitrate was significantly ($p < 0.001$) higher in the conventionally grown spinach compared to the organically grown spinach (Table 2). The response of spinach cultivars varied with a maximum increase of 4-fold (Figure 4). Spinach is particularly responsive to soil nitrogen levels because of a very efficient uptake system and inefficient reduction systems.[34] Of the 27 spinach cultivars studied, 14 conventional cultivars contained more than 100 mg of nitrate/100 g of FW.

The accumulation of oxalate in spinach depends upon variety, fertilizer application, light intensity, and growing season.[18,28,31,32] However, herein cultivation had no significant effect on the oxalate content of spinach (Table 2 and Figure 5).

Correlation between Ascorbic Acid, Vitamin C, Flavonoids, Nitrate, and Oxalate. Total flavonoids correlated positively with ascorbic acid and vitamin C, whereas nitrate correlated negatively with ascorbic acid, vitamin C, and total flavonoids (Table 4). These are in good accordance with the carbon/nutrient balance theory and growth rate and growth-differentiation balance hypothesis, indicating the allocation of plant metabolism toward higher carbon-containing components (ascorbic acid and flavonoids) and lower nitrogen-containing compounds (nitrate). Kaminishi and Kita found a moderate negative correlation between nitrate and oxalate in spinach.[18] Interestingly, in this study, a slight positive correlation was observed between these components, similar to the result by Elia et al. demonstrating that oxalates increased with a higher nitrate concentration to limit soluble organic anion content produced during nitrate reduction.[31] Kaminishi and Kita compared 182 spinach varieties grown in a open-sided vinyl house over four growing season (summer, fall, winter, and spring), and the levels of nitrate and oxalates showed distinct seasonal variation. In our study and the study by Elia et al., hybrid varieties were grown in only one season. These differences may account for the correlation differences found between nitrate and oxalate between these studies. Some components, including ascorbic acid and flavonoids, have been reported to inhibit the toxic effects of nitrites.[8] Such findings suggest that organic spinach containing higher ascorbic acid and flavonoids but less nitrate content would be more health-promoting than the conventional counterpart.

In conclusion, this study demonstrates that the levels of ascorbic acid and flavonoids are significantly higher across 27 spinach varieties grown by organic cultivation methods compared to conventional practices. Conversely, the content of nitrate was significantly higher in the conventionally produced spinach compared to the organically grown spinach. Total flavonoids correlated positively with ascorbic acid and vitamin C. Nitrate correlated negatively with ascorbic acid, vitamin C, and total flavonoids. These results suggest that organic cropping systems result in spinach with higher levels of flavonoids and ascorbic acid and lower levels of nitrates.

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Notes

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